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BORDERLINE AND LOCALLY ADVANCED PANCREATIC CANCER – REDEFINING THE BIOLOGICAL AND TECHNICAL PROFILE OF THE DISEASE

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Borderline and locally advanced pancreatic cancer – redefining the biological and technical profile of the disease

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Stockholm 2019

Aut viam inveniam aut faciam.

Dedicated to bring hope to those who fight pancreatic cancer. And to those who fought.

ABSTRACT

Surgery is the only treatment modality that provides a chance for long-term survival in pancreatic cancer (PC). Thus, the current classification systems for PC are technically skewed to predicted the probability for surgical resection, and not adapted for tumor biology. As more potent oncologic therapy steps forward, questions arise whether more aggressive surgery is motivated and how to select the better surgical candidates based of predicted tumor behavior. Also, new tumor-specific treatments should be sought to overcome the aggressive PC biology.

Paper I investigated the short and long-term outcome in a series of pancreatectomy with venous resection (VR). VR can be carried out safely, with low morbidity attributable to the vascular reconstruction itself. No factors were associated with severe morbidity. VRs brought similar survival benefit for resectable, borderline (BRPC) or locally advanced (LAPC) or type of periampullary tumor. Factors pointing shorter survival were attributable to tumor biology and patients' characteristics (elevated CA19-9 and ASA score) and not technical in nature.

Paper II investigated the role of surgery after neoadjuvant treatment (NAT) for BRPC and LAPC. Surgical resection could be carried out safe, despite that vascular procedures were most often required. Surgery significantly improved survival both after FOLFIRINOX and other combination chemotherapy, even for higher levels of preoperative CA19-9. Even significant dose reductions of FOLFIRINOX did not impair the prognosis. There was no difference in survival between BRPC and LAPC patients, whether resected or not, and the recurrence pattern was similar - with distant metastases in all and few local recurrences.

Paper III looked at the impact on survival of biologic prognostic factors potentially available preoperatively (mGPS, CA19-9, para-aortic lymph node, PALN, status) in resected patients with resectable, BRPC, and LAPC. All factors could much better discriminate differences in survival than the resectable, BRPC, and LAPC, including inside each category. Positive PALN had strongest negative impact on survival; their presence was significantly associated with elevated preoperative CA19-9, particularly in LAPC patients after NAT.

Paper IV found that tumor-infiltrating lymphocytes (TILs) can be isolated from PC in sufficient amount required for adoptive transfer therapy. TILs showed phenotype that can expand upon stimulation, home, and recognize tumor-associated antigens and autologous tumor cells, and induce autologous tumor-cell killing in culture.

In conclusion, new classification of PC is needed that better reflects the chance for survival. Biological factors should be integrated to successfully guide treatment, even if would still have leading role. Possibilities open to target specific tumor biology by adoptive TIL transfer.

LIST OF SCIENTIFIC PAPERS

I. **Rangelova E**, Valente R, Kivila R, Tanaka K, Halimi A, Arnelo A, Segersvärd R, Del Chiaro M.
Technical and oncologic aspects of venous resections during pancreatectomy – whom and how to resect?
Manuscript

II. **Rangelova E**, Wefer A, Persson S, Valente R, Tanaka K, Orsini N, Segersvärd R, Arnelo U, Del Chiaro M.
Surgery improves survival after neo-adjuvant therapy for borderline and locally advanced pancreatic cancer: a single institution experience.
Ann Surg 2019; Apr 2. doi: 10.1097/SLA.0000000000003301. [Epub ahead of print]

III. **Rangelova E,** Tanaka K, Valente R, Halimi A, Löhr M, Arnelo A, Segersvärd R, Del Chiaro M.
Preoperative risk factor stratification gives better estimate of survival in resected patients than current classifications of borderline and locally advanced pancreatic cancer.
Manuscript

IV. Meng Q*, Liu Z*, **Rangelova E***, Poiret T, Ambati A, Rane L, Xie S, Verbeke C, Dodoo E, Del Chiaro M, Löhr M, Segersvärd R, Maeurer MJ.
Expansion of tumor-reactive T cells from patients with pancreatic Cancer
J Immunother. 2016 Feb-Mar;39(2):81-9.

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LIST OF ABBREVIATIONS

α-SMA	Alpha-smooth muscle actin
ASA score	American Society od Anesthesiologists score
BRPC	Borderline resectable pancreatic cancer
CA	Coeliac axis
CA19-9	Carbohydrate Antigen 19-9
CHT	Chemotherapy
DP	Distal pancreatectomy
IPMN	Intraductal papillary mucinous neoplasms
IVC	Inferior caval vein
LAPC	Locally advanced pancreatic cancer
LNR	Lymph node ratio
MDSCs	Myeloid-derived suppressor cells
mGPS	Modified Glasgow Prognostic Score
NAT	Neoadjuvant therapy
PALN	Para-aortic lymph nodes
PanIN	Pancreatic Intraepithelial Neoplasms
PC	Pancreatic cancer
PD	Pancreaticoduodenectomy
PDAC	Pancreatic ductal adenocarcinoma
PV	Portal vein
PSC	Pancreatic stellate cells
RECIST	Response evaluation criteria in solid tumors
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
TAA	Tumor-associated antigen
TIL	Tumor-infiltrating lymphocytes
TME	Tumor microenvironment
TP	Total pancreatectomy
VR	Venous resection

TABLE OF CONTENTS

1. Introduction

Pancreatic cancer (PC), is not among the most common cancers worldwide and in Sweden (incidence of about 1,100 and 1,400 cases per year)(1-3), but is one of the leading causes of cancer-related death with an estimate that by 2030 it will climb up from 4th to 2nd place (2, 4, 5). While the prognosis for the majority of cancers have progressively improved over the decades, usually parallel to the investment in research, the prognosis for PC patients remains dismal (6, 7). The estimated 1-year survival for all stages of the disease is about 18% and the 5-year survival about 4-7 %, as number of the deaths of PC lies close to the number of the newly diagnosed cases , meaning almost everybody affected will die from the disease (3, 8). A major reason for this phenomenon is that the diagnosis generally comes too late –due to very low disease awareness and late turn for help, but also because the disease progression is often asymptomatic (9-11). More than half of the patients with PC present with metastatic disease, with expected survival between 3 and 6 months (12). Another 30% do not have distant metastases, but locally advanced pancreatic cancer (LAPC), engaging the peri-pancreatic vessels (celiac, hepatic arteries, SMA, PV/SMV), where radical surgical resection is traditionally not regarded as possible (13). Only about 15-20% of patients diagnosed with PC present with localized tumors where radical surgical resection can potentially provide the only chance for cure. Even after resection with curative intent, though, the median survival is less than 2 years, meaning that the majority of patients will develop disease recurrence and only about 20-25% will survive 5 years. This hints for the second major reason for the dreadful prognosis of PC, that is the unfavorable tumor biology. PC has a unique microenvironment allowing for its aggressive local growth and early development of metastases at the same time as showing extraordinary resistance to chemo- and radiotherapy and current targeted therapies(14).

Some slight improvement in the prognosis of PC have been observed worldwide, mostly after 2010. The National Cancer Institute reports improvement in 5-year survival from 3-4% to 9.3%, while in Sweden the increase is from 4 to 8.3% (3, 15). Patients with oncologically treated LAPC and metastatic PC are still unlikely to pass the 5-year survival time-point(16). Patients with resectable PC, however, exhibit better chance for 5-year survival due to improvement of surgical technique and safety, followed by combination adjuvant chemotherapy, reducing the risks for cancer recurrence (17-20). This “lift up” in survival is most probably attributable to the better survival odds for this small proportion of patients, but

this positive trend is, unfortunately unable to provide a major turnover of the dreadful prognosis of PC.

Currently, there is no effective primary prophylaxis, no screening programs for the general population, and no biomarkers for early detection of PC (21, 22). Few life-style factors have been linked to increased risk of PC (smoking, alcohol, obesity, diabetes, dietary preferences), but without too strong correlation to be used as prevention tool (21, 23-27). Some genetic syndromes and hereditary pancreatitis have also been linked to increase the risk for PC (Peutz-Jeghers syndrome, PRSS1 mutation, familial atypical multiple mole melanoma, BRCA1/BRCA2 mutations, HNPCC (20, 21, 28-30) as well as familial pancreatic cancer (without common genetic mutations) where the risk increases exponentially to the number of the first-degree relatives with PC (21, 28). Knowing the relative risk for developing PC in these conditions allows for selective high-risk individual screening for possibly early detection and timely surgery(31-33). However, these represent not more than 10-15% of the diagnosed cases (28, 31-33).

Early detection and treatment of precursor lesions have increased the probability for survival, for instance for breast and colorectal cancer. There are two known types of precursor lesions to PC with a well described pathway of progression from dysplasia to invasive cancer – pancreatic intraepithelial neoplasms (PanIN) and intraductal papillary mucinous neoplasms (IPMN) (29, 33-37). PanIN is a microscopic epithelial lesion, usually arising in the smaller ducts, with a clear progression pattern to PC, although this rarely happens –approximately 1% life-long (29, 38). It is hardly detectable by current imaging modalities and therefore not suitable as a screening target for prevention (29, 39). IPMN, on the other hand, are mucin-producing lesions that cause dilatation in the pancreatic ductal system and in this way can be visualized on standard imaging – at best on magnetic resonance imaging (MRI) and EUS(40, 41). Distinct risk factors for malignant transformation have been identified – dilatation of the main pancreatic duct, enhancing mural nodules, elevated serum Ca 19-9, symptoms arising from the pancreas, but there is no clear cut to differentiate when the low-to-high-grade dysplasia transformation and invasive cancer occurs, which would indicate the best timing for resection (42-44). Whenever these risk factors are identified in fit individuals, surgical resection is recommended to rule out early cancer or preferably prevent further dysplastic progression to invasive cancer (33, 42). Few guidelines are dealing with the indications for follow-up and surgical resection (42-44). Generally, more liberal indications for surgery, with

focus on prevention, are considered in young and fit patients with long life-expectancy. In less fit individuals more sinister feature or constellation of worrisome features are sought before considering surgery, due to the difficult balance with the burden of complications after pancreatic surgery in these patients(42). As the risk for progression continues beyond 5 years of observation, so life-long surveillance is indicated until the individuals are fit for surgery (37, 42, 45). In this manner the cost burden for health care need to be better addressed and less costly surveillance modalities sought (46, 47). Whether addressing and treating IPMN will result in actual decrease in the incidence of PC and improve the overall prognosis of the disease is, though, at this point still not known.

GLOBOCAN estimates that by 2040, the incidence of new PC cases will increase by almost 80%. If a break-through in the treatment of PC does not occur soon, the burden of PC for health care and society will progressively increase. Two factors can currently be addressed to further improve the prognosis. One of them is to optimize the applicability of the best treatment method – surgery. More aggressive surgical procedure prove to be feasible. It is essential, though, to better select the "good" surgical candidates, who would benefit from the local treatment that surgery is. Increasing the pool of patients suitable for surgery and converting the "poor" to "good" candidates, possibly by multimodality treatment, will potentially raise the odds for survival of PC. To do this, it is critical to better understand the risk for disease progression and stratify the patients according to the expected disease aggressiveness, that now lies beyond the scope of what the current classification systems can cover. The second factor is to urgently look for treatment options different than the standard oncologic modalities, that repeatedly show limited efficacy. Understanding better the biology and modifying the host own possibility to control the disease, by its own defense mechanisms, amplified by for instance immunotherapy, might be a step in the right direction. The current thesis deals with both of these issues.

2. Background

2.1. Basics of tumor biology of pancreatic cancer

2.1.1. Mutations, progression, and metastases in PC

PC follows a step-by-step carcinogenesis with accumulation of mutations – from founder to progressor mutations, until a clone carrying metastatic potential occurs. This process requires more than a decade to occur(34, 48). The metastatic portfolio of PC has been described to carry more than 2000 mutations (48, 49), but the mutational burden of a single genome (i.e. single patient) is on average of 26 mutations (ranging from 1 to 116)(49). Four of the mutations are considered hallmarks of PC, being present in vast majority of pancreatic ductal adenocarcinomas. These are *KRAS* (in >90%), *CDKN2A, TP53* and *SMAD 4* (in 50—80%) (30, 48-50). In this order they are gradually encountered already in precursor lesions during progression of dysplasia - from PanIN-1 to PanIN-3(34, 48). The loss of *SMAD4* correlates in animal models and clinically with high metastatic burden (48, 50, 51). After the metastatic clones occur, all metastatic herds afterwards bear the signatures of the latter. This model can explain why targeted therapies usually fail – addressing a genetic alteration target its associated subclones, but fails to influence the preceding clones that lack the target and that could still take over and expand(48).

Four subtypes of PC have proposed based on their genomic variation(30, 49):“stable” (<50 structural variants, in 20% of cases.), “locally rearranged” (large number of events, localized onto 1-3 chromosomes, in 30%), “scattered” (50 - 200 events , in 36%), and “unstable” (>200 genomic structural variants, in 14%). This subtyping correlates to possible response to therapy – the unstable subtype with its DNA maintenance defects has been regarded as a potential biomarker for sensitivity to platinum agents and PARP inhibitors (52).

The unfavorable outcome even after radical surgical resection is probably explained by the fact that PC is usually a systemic disease even in apparent earlier stages. Recent animal studies show the worrisome observation that metastatic dissemination can occur even before the primary tumor becomes clinically evident – neither by imaging nor by dedicated histologic examination(53). During the process of tumorigenesis, already at the stages of dysplasia (PanIN, harboring only KRAS mutation), epithelial-mesenchymal transformation

(EMT) and migration through the basal membrane and into the circulation can occur, before the truly invasive cancer has been formed (50, 53). The cells undergoing EMT and entering the circulation generally maintain their mesenchymal phenotype and express features resembling cancer stem cells, with the ability for self-renewal, survival and tumor infiltration (50, 53). Circulating PanIN cells with mutant *Kras* are though hardly capable of colonization of distant sites until critical mutations accumulate – in Trp53 and or $p16^{Ink4a}$/arf genes (50). This metastatic competence generates during the process of tumor expansion and thus microscopic metastases can be present even in small tumors. That explains the common clinical presentation and the inability of the current diagnostic methods to accurately predict the right stage of disease (50).

2.1.2. Tumor microenvironment (TME) of pancreatic cancer

PC is characterized by abundant stroma (or TME) and poor cellularity – about 38% (5 to 85%) compared to other cancers(14, 49). The stroma serves both as a mechanical barrier that blocks drug penetration, but also has an active biological role. The TME is composed of blood vessels, immune cells, pancreatic stellate cells (PSCs), fibroblasts, cytokines, growth factors, extracellular matrix, etc and evolves throughout the progression of PC(14).

The PSCs , delineating the acini, get activated under inflammatory conditions to abundantly produce and deposit extracellular matrix and express specific markers (α-SMA) (14). This mechanisms in the early stage of tumorigenesis may have a protective role against PC, by defining host's attempt to isolate cancer cells, but in the later phases exhibits pro-tumoral properties(30, 54). PSCs are able to migrate to distant metastatic sites, inhibit apoptosis and enhance survival of PC cells, facilitating the creation of cancer stem cell niche and thus seeding of PC cells (30). Cancer-associated fibroblasts are insensitive to chemotherapy compared to epithelial cancer cells and build shield-like structures around the latter, thus "guarding" them from exposure to chemotherapeutic agents(55).

The extensive extracellular matrix, generated by PSCs, changes the tissue architecture and configuration of the blood and lymphatic vessel networks(14, 56). Unlike other solid tumors, a typical feature of PC, is its poor vascular network, in contrast to the previous assumption that an "angiogenic switch" is necessary for tumor progression(14, 50, 57). The increased distance between the blood vessels and cancer cells hampers the diffusion of drugs through

the tissue and the drug concentration at the target cancer cells falls under the therapeutic levels (58). It destines all drugs, irrespective of their efficacy *in vitro*, to fail due to their inability to reach the target. This is likely the reason for the observed striking discrepancy between the successful investigational therapies observed in vitro and the largely disappointing results in clinical trials (14, 59). Treatment strategies focusing on combining drugs that increase the vasculature or break down the surrounding blocking desmoplastic reaction are tested together with chemotherapeutic agents – up to this point without promising results(14, 60). The poor blood supply in combination to the higher energy demand of cancer cells creates a hypoxic environment. Hypoxia, so inheritant to PC(56, 61), operating through hypoxia inducible factor (HIF) and Notch signaling pathways, increases the invasive and metastatic potential of cancer cells, improves their resistance the chemo and radiotherapy and creates features resembling these of cancer stem cells (14, 62).

2.1.3. Immune system in PC

2.1.3.1. Inflammation

Inflammation is one of the hallmarks of cancer(63). PC is no exception, being typically characterized by both local and systemic inflammation. Induction of inflammation (pancreatitis) promotes EMT transformation, invasion and dissemination into the circulation and has been associated with more advanced stages of PanINs (53). Inflammatory signals induce cell reprogramming and susceptibility of pancreatic epithelial cells to *Kras*-driven neoplastic transformation (50, 53). The expression of cyclooxygenase-2 (COX-2) is undetectable in normal pancreatic tissue, but rises during the PanIN progression, and reaches 90% in human PDACs, delineating its role in carcinogenesis (64, 65). Treatment with anti-inflammatory drugs, such as dexamethasone or non-steroidal anti-inflammatory drug, NSAID (nimesulide), reduces PanIN formation and the amount of circulating EMT transformed cells (53, 66-68).

IL- 6 plays a central role in the systemic inflammatory response observed in PC, being expressed by multiple cell types in the TME (macrophages, fibroblasts, endothelial cells, myeloid cells, immune cells), as well as by PC cells themselves(69-71). It shares the responsibility for the initiation and maintenance of precursor lesions, for immune evasion,

resistance to apoptosis and cancer progression(69, 70). Its secretion by non-cancer cells promotes the formation of pro-metastatic niche in the liver(72). In humans, elevated IL-6 levels in serum and tissue have been associated with increased resting energy expenditure, development of cancer cachexia and poor prognosis(73, 74). Other cytokines (IL-8 and IL-10) have shown the same link, as the elevation of more than one of these cytokines has been inversely proportional correlated with survival(73, 75). IL-6 is a regulator of the hepatic acute phase protein response, during which a variety of mediators that initiate and sustain systemic inflammation are released into the circulation (74). Among these, C-reactive protein (CRP) and albumin have most frequently been addressed in predicting survival in patients with various advanced cancers, including PC (75-79).

a. Measuring systemic inflammation

Prognostic scores, using different components of the inflammatory response, have been created in an attempt to capture the effect of systemic inflammation on cancer-specific prognosis. The neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, lymphocyte-monocyte ratio, and Glasgow Prognostic Score (GPS) have all been linked to predicting PC survival (80-85). Among the generally encountered difficulties in using all these scoring systems are the confounders, not unusually observed, that could influence their prognostic significance (e.g. ongoing pancreatitis or biliary obstruction, particularly relevant for PC). Being able to correct for these issues, increases the prognostic significance of the scores(86).

GPS is probably the most well studied and most easily available of the inflammation scores. It is built up by correlation between serum CRP and albumin (84, 85). GPS scores of 0,1, or 2 are appointed depending on whether any of the two deranged parameters (CRP > 10 mg/l or albumin <35 g/l) are being present – neither of them (score 0), only one (score 1), or both (score 2). The modified GPS (mGPS), that is currently more widely used, gives more weight to elevated CRP since it was shown to have stronger correlation with prognosis. The mGPS scores 0, 1, and 2 are as follows(87):

Score 0: CRP ≤ 10 mg/l, any albumin
Score 1: CRP > 10 mg/l, albumin ≥35 g/l
Score 2: CRP >10 mg/l, albumin <35 g/l

GPS has been able to make survival prediction in several cancers (colorectal, oesophageal, gastric, lung, cholangiocarcinoma, urologic and gynaecologic cancers), irrespective of the clinically diagnosed stage – localized or advanced, indicating that it captures a different perspective of tumor biology than what imaging can show (74, 78, 80, 84, 85, 88). In PC, increased GPS has been correlated to poor tumor infiltration with immune cells and shorter survival(89). PC patients with mGPS of 1 or 2 were shown to have worse expected survival and are more likely to have metastatic disease compared with patients with mGPS of 0 (87, 90-92). mGPS was hinted to have better predictive value in more advanced tumor stage (90, 93). Postoperative higher GPS of 1 and 2 after curative resection, measured before the start of chemotherapy, was also predictive for worse survival(94).

The prognostic value of GPS has been evaluated also particularly for BR/LAPC. Pretreatment higher GPS was predictive for survival after chemoradiotherapy for LAPC (95). Similar results were also reported previously for esophageal cancer, though neoadjuvant treatment was followed by surgery(96). In a series of 38 patients with conversion surgery after neoadjuvant chemo(radio) therapy for LAPC and metastatic PC, preoperative GPS of 0 (but CRP cut-off set to ≤ 5 mg/l) was found to be an independent prognostic factor for long-term survival, stronger than CA19-9 decrease (97). Whether GPS should be used as criteria to select which patients should benefit from surgery in BR/LAPC after neoadjuvant therapy, need to be validated in larger and more homogeneous cohorts.

Combining elevated GPS (1 or 2) with elevated Ca19-9 ($\geq$180 U/ml) has shown an even better prediction of the risk for early recurrence after surgery and adjuvant chemotherapy for PC(98). The measurements, though, took place before the start of chemotherapy. Whether these could be used to predict the benefit of resection in BR/LAPC before the decision for surgery has been taken, is unknown.

b. Treating inflammation

In colorectal cancer, preoperative treatment with NSAID was correlated to increased tumor infiltration with immune cells (99). Such observation in PC is lacking, However, leaning on data from preclinical studies(53, 67, 68, 100), modulation of the systemic inflammation

response by both selective and non-selective NSAIDs has been studied in PC (101, 102). Just a week of treatment with Ibuprofen in weight-losing PC patients was able to reduce the resting energy expenditure and the CRP levels (103). Furthermore, survival of patients with metastatic cancer almost doubles(104). Whether this fact could influence the final cancer prognosis in PC still remains to be determined.

2.1.3.2. Adoptive immunity

During carcinogenesis, cross-talk between the tumor and the immune system takes places, responsible for the three phases of cancer *immunoediting*: 1. *elimination phase* (early stage of immune surveillance that may lead to tumor elimination); 2. *equilibrium phase* (the immune system edits the tumor immunogenicity); and 3. *escape phase* (tumor cell subpopulations not effectively recognized, re-directing the immune system to tumor-supportive phenotype) (105, 106). The immune cell populations and immune mediators progressively increase and change throughout all stages of development of PC, from inception from precursor lesions to invasion, helping the tumor to progress and increase its aggressiveness(70, 107, 108). The "good" local inflammation in the initial stages of carcinogenesis, having better effector functions against the transformed epithelial cells (containing $CD8^+$and Th1 $CD4^+$ T lymphocytes, NK cells, mature dendritic cells (DC), type M1 macrophages, IL-2, TNF-α, IFN-γ), gradually evolves into ineffective "bad" inflammation, sustaining the cancer growth (with regulatory T-lymphocytes, MDSCs, M2 macrophages, ineffective CD8+ lymphocytes, immature DC, TGF-β, IL-10)(108, 109). Interestingly, tumor cells themselves actively participate in the immune modulation (acting themselves as "immune cells") by expressing inhibitory signals (such as TGF-beta, IL-10 and IL-6, VEGF, PD-L1 and Foxp3, Fas-L), co-stimulatory molecules (B7-H3, CD40, CD40L) or by down-regulating the expression of antigens(109-111).

In PC in particular, stromal reaction is present from the very early PanIN stages of tumor development, with recruitments of immune cells, including immunosuppressive populations ($Foxp3^+$ $CD4^+$ T_{reg} lymphocytes, tumor-associated macrophages, TAMs) (70, 107). Thus tumor-infiltrating lymphocytes (TILs, $CD3^+$), almost never present in normal pancreata, become more pronounced during PC progression, evolving from predominantly $CD4^+$ in preneoplastic lesions to both $CD4^+$ and $CD8^+$ in invasive cancer, but with grossly varying density of infiltration. The majority of TILs (85% in mouse models) seemed to be

represented by naïve CD8+ T cells – non-activated and not recognized an antigen ($CD45RB^{high}$, $CD44^{low}$)(107). PSCs and cancer-associated fibroblasts (CAFs) in PC may aid in immune evasion by sequestering of the $CD8^+$ T cells in the stroma and by induction of expression of inhibitory markers on them (30, 112). The presence of MDSCs almost excludes the presence of $CD8^+$ T cells and $CD8^+$ T cells are regarded as the main effector in the anti-tumoral responses (107).

a. Immunogenicity

What determines a tumor's immunogenicity is the expression of sufficient number of unique antigens (in relation to the original normal cells) that are effectively presented by the tumor via MHC complexes so that a full immune response can be induced(113, 114). Expression of weak antigens or inadequate presentation leads to weak immune responses – due to reduced expression of "danger signals", so that antigen-presenting cells do not get sufficiently activated, or by further modifying the immune response by inhibitory signals. Hence, immune tolerance instead of elimination takes place, which aids the tumor cells to develop evasion mechanisms, gain power and progress(113).

PC is known to be poorly immunogenic. The low prevalence of somatic mutations in PC is generally considered to be a premise for failure of immune therapy, because fewer immune targets are available (115-117). PC antigens were also shown to generate relatively weak immune responses (115). Despite that, a variety of tumor-associated antigens (TAAs) have been found in pancreatic cancer that could be used as a potential target for therapy – mutated K-ras, MUC1, hTERT, CEA, surviving, p53, HER-2/neu, GAGE, mesothelin, SCP-1, SSX-4, HERV-K-MEL, MAGE-A1&2, NY-ESO-1 (114, 115, 118-122).

b. Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) have been closely related to the survival outcome in a variety of tumors. $CD8^+$ TILs are considered as the main effector arm of the tumor response (123), but $CD4^+$ T cells were also shown to be able to elucidate anti-tumor responses(124). A progressively increasing number of reports confirms the strong prognostic role of infiltrating TILs in PC, and particularly $CD8^+$ TILs(89, 125-131). In a series of resected patients, on

immunohistochemistry staining for CD4 and CD8, the simultaneous tumor infiltration with both $CD4^+$ and $CD8^+$ TILs was found to be an independent prognostic factor for better survival (129). The reported 5-year survival was 48.4%. This impressively beneficial coexistence, together with the finding that also dendritic cells (DCs) tend to be present in these cases, is a reflection that an effective antigen recognition and adequate cellular cross-talk takes place. Thus, the responsiveness of the immune system towards the tumor seems to play a substantial role for the cancer control. Co-location with B-lymphocytes was also shown to have a more pronounced effect for prolonged survival(126, 130). The survival of patients was reported to be better whenever strong infiltration of $CD8^+$ TILs and expressing PD-1 TILs was present – showing that PD-1, besides being an inhibitory marker, could also represent that experience and activated TILs, recognizing a tumor target, are present (127, 132). PD-1 expression could also be used as a possible predictive marker for success for eventual check-point inhibitor therapy(127).

The prognostic significance of the location of TILs – inside the tumor or in the periphery, does not seem to be as distinct in PC as in, for instance, colorectal cancer(89, 126, 127). Whether intraepithelial or stromal TILs are more important is also uncertain (126, 128, 130). Not unusual, though, $CD4^+$ and $CD8^+$ TILs are observed captured in the stromal tissue, far away from cancer cells and lacking the expression marker of memory cells, *CD45RO* (133).

Other immune cells in the tumor environment have impact on the cancer prognosis, too (B lymphocytes, NK cells, TAMs, neutrophils)(105, 126, 131, 134, 135). The correlation among the different immune cell types has an even stronger prognostic significance than the single populations, as it reflects the anti-tumoral effectiveness of the immune reactions(131, 136). Stronger infiltration with CD4+, CD8+ TILs, DCs is associated with better survival, while the survival is poor if the prevailing proportion constitute of $FOXP3^+$ Tregs, M2 TAMs or MDSCs (129, 131, 132, 136, 137).

The composition of the immune-cell infiltration retains its role for prediction of prognosis even in PC pretreated with neoadjuvant therapy(136). High $CD4^+$ and $CD8^+$ T cells infiltration, particularly when fewer $FOXP3^+$ T lymphocytes are present, predicts better chances for survival. Interestingly, successful neoadjuvant therapy might be able to tip over the balance among immune cells populations by reducing the proportion of Tregs and MDSCs(132).

2.1.3.3. Immunotherapy

Immunotherapy is most often based on the principle of recruiting and activating T cells that are able to recognize tumor antigens and induce effective cytotoxic response against them. Other mechanisms are also being investigated, such as designing recombinant monoclonal antibodies to target tumor-specific antigens and induce cell death by direct lysis or through delivery of a conjugated cytotoxic drug.

There is steadily an increasing amount of publications focusing on different immunotherapeutic agents (71). Most of the trials are focused on boosting the immune system with peptide vaccines in patients with metastatic PC, but failing to show effect on survival. GVAX, combining two allogenic tumor cell lines and an adjuvant, did succeed to demonstrate some clinical benefit in resectable and metastatic disease, but, unfortunately, without impressive results so far (76, 77). Some clinical effects have been observed with dendritic cell vaccines, too, given systemically or by intratumoral injections (79, 80).

The best effects with immunotherapy so far have been observed with adoptive cell transfer therapy of TILs. In patients with metastatic malignant melanoma, objective responses were observed in 72% of patients when TILs were administered after pre-conditioning chemotherapy with cyclophosphamide and fludarabine and whole-body radiation (81). In the patients who were found to be complete responders (22% of patients), the 3- and 5-year survival was 100% and 93%, respectively – results that have not been achieved with any other type of oncologic therapy.

By standard approach, the long-term survival in patients with PDAC can be improved, but still the majority of patients will die from the disease. A conceptually different approach, like immunotherapy, is therefore mandatory. For the disease that PDAC is, the most potent treatment should be sought to possibly generate the best possible outcomes. Unfortunately, the isolation of TILs in PDAC has been cumbersome and not successful up to the point of the current research.

2.2. Pancreatic surgery

Pancreatic surgery has evolved since the introduction of pancreaticoduodenectomy more than a century ago by Codevilla and later on by Kausch and Whipple(138, 139), and developed further by Fortner and Traverso and Longmire(140, 141). The unacceptably high morbidity and mortality rate in the beginning of the 20th century has led to its abandoning for a while, before it was picked up again with increasing popularity in the 70s(140). Since then, much has happened in terms of surgical safety. Nowadays the 30-day mortality should be less than 5%, most often around 1-3% in large centers (142, 143). The centralization of high-risk relatively rare surgery, like pancreatic surgery, into fewer centers and fewer hands (high-volume centers) has led to substantial improvement, not only of the short-term morbidity, but also of the long-term prognosis of the disease(20, 144-146). This effect allows also for more aggressive and complex surgical procedures to be carried out with better patient outcome(147, 148). This development helps to challenge even the long-lasting taboos of what is technically possible to achieve and confront even a disease like PC on a different level.
The burden of surgical morbidity, is however still present, most often attributable to the leakage from the pancreatic anastomosis. Thus, the benefit of surgical resection still needs to be evaluated parallel to the estimation of the surgical risks.

Pancreatic surgery is currently the only treatment that can offer a chance for cure, although to a small proportion of the patients with PC. The reason is the challenging anatomic location of the pancreas, closely adjacent to major abdominal vessels, enabling their early encasement by a growing tumor. This leads to the not infrequent narrow resection margins (>80%) after resection, with microscopically residual disease (R1) within 1 mm of the resection margin (149, 150). Although the broader the tumor-free margin is, the better the outcome has been reported (151, 152), due to the tricky anatomical position of the pancreatic tumor, for the majority of cases this is unfortunately unachievable and non-realistic. Besides, even if R1 margin status is associated with higher risk of developing local recurrence, the natural history of disease reveals that distant metastases develop before the local recurrence becomes apparent. That fact also implies that occult metastatic disease might be more prevalent than estimated even in cases of radiologically defined localized disease and is the reason for disease recurrence and failure of the attempt for cure by surgical resection (153, 154).

2.2.1. Borderline and locally advanced pancreatic cancer

If the tumor is extending beyond the pancreas and involving the celiac axis and the superior mesenteric artery, it is regarded as locally advanced, or staged as T4 according to the TNM classification of Malignant Tumors (TNM) (155). In this manner, it is considered that the tumor cannot be radically removed by standard surgical resection and therefore treatment with curative intent is doubtful to be successful.

The concept of borderline resectable pancreatic cancer (BRPC) was introduced in 2005 by Varadhachary *et al*, implying that the tumor could theoretically be resected, but positive surgical margins could be expected and therefore neoadjuvant oncologic therapy might be advisable. Radiologically, BRPC is described by involvement of the porto-mesentheric venous axis and different degrees of involvement of the hepatic arteries, SMA and the celiac axis. The exact definition of what is considered BRPC varies in literature, which hampers the comparability of the published studies. Among the most widely considered definitions are the updated NCCN definitions (156), the MD Anderson definition (157-160), the ISGPF consensus statement (161), the international consensus by IAP (162)(36). Generally, what the definitions have in common, for the sake of general applicability, is the consideration that radical surgical resection can be performed by standard pancreaticoduodenectomy procedure(138, 163), possible with PV/SMV resection, but not necessitating arterial resection and reconstruction(164). Interestingly, regarding the PV-SMV involvement in BRPC, despite that the concept of "borderline" resectability implies possible advantage of neoadjuvant treatment, at present there are limited number of high-evidence trials confirming that patients benefit from upfront oncologic therapy (161, 165-170). That the "borderline" PC group is constantly attempted to be defined, is due to the fact that there is a group of patients who, even not resectable by standard operation, might benefit from radical surgical procedure and also obtain a chance for cure. Whether the current geometric description of vessel involvement really corresponds to tumor biology or a better discriminator of unresectable disease needs to be approached, still needs to be defined, particularly in the era of more potent chemotherapeutic drugs.

2.2.2. En bloc vascular resections during pancreatic surgery

The first reports on the resection of the peri-pancreatic vessels (PV-SMV) date from the middle of the 20th century, and being termed by Fortner in the 70s as "regional pancreatectomy" (140, 171, 172). After a short while, the technique was abandoned due to unacceptably high morbidity and mortality rates without an apparent survival advantage. With the current advancements in peri-operative care and particularly after FOLFIRINOX came into the spotlight, the interest towards more aggressive approach has been reborn.

2.2.2.1. Venous resections during pancreatectomy

Resections of the PV-SMV during pancreatectomy are at present considered standard of care by some of the large-volume pancreatic centers (142, 143, 159, 161, 173-180). Two meta-analyses show that PV-SMV can be performed with reasonable and comparable morbidity to standard resections and that the long-term oncologic outcome is favorable (181-183). Despite that, there is still concern regarding the burden of complications related to the venous reconstruction and reluctance to perform venous resection in many HPB centers – due to high reoperation rates and risk for thrombosis or loss of patency over time (142, 177, 183, 184). Thrombosis rates differ in the early and late postoperative period – early being reported as 7.5-13.3% and late - as high as 26.7% have been observed months to years after surgery – most often associated with recurrence and being symptomatic in the majority of patients (177, 183, 185, 186). Venous thrombosis has been reported to occur more often in the setting of neoadjuvant therapy and when interposition grafts are used – autologous and particularly prosthetic (185, 187-189). However, the results are contradicting whether the latter are associated with impaired long-term prognosis(185, 187, 189, 190).

With centralization of surgery, there is continuous improving of the perioperative results, and therefore studies involving older time periods in order to increase the number of reported patients, might draw irrelevant nowadays conclusions. For instance, studies reporting perioperative outcome of venous resections from the same database (NSQIP) report different outcome if short recent period is viewed (2014 and 2015 – no difference in morbidity and mortality to standard PD) (176), compared to increased complication and mortality rate if earlier periods were included (2005 to 2009)(191).

Another unclear issue is how PC with PV-SMV involvement should be classified – as primary resectable or borderline resectable. Besides, in the current definition consensus statements, the

cut-off between BRPC and LAPC with venous engagement is generally described as being able to obtain sufficient length of PV and SMV that is necessary for safe reconstruction (156-158, 161, 162). It is generally accepted that up to a maximum 2-3 cm of PV-SMV can be resected, if a graft interposition needs to be avoided, otherwise sleeve resection is performed with unavoidable stenosis of the lumen(184, 185). The technical possibility for reconstruction is a subjective parameter dependent not the least on the surgical performance and expertise and not necessarily a sign of biologically advanced disease. For instance, a small tumor, unfortunately located at the root of the mesentery and engaging the first jejunal branch would most often be regarded a LAPC, while a tumor widely docking onto and surrounding the PV-SMV in its middle portion, and possibly extending more into the retroperitoneal space, would be at most BRPC. Currently, at large-volume centers resections of the root of the mesentery with sacrifice of the first branches of the SMV can be performed with no different outcome, while after extensive mobilization of the root of the mesentery *ad modum* Cattell-Braasch, the whole venous axis (even 7 cm) could be resected even without graft interposition (174). Keeping the reconstruction simple, by a single anastomosis, shortens the cross-clamping time and avoids venous congestion of the bowel with potentially decreasing the risk of complications(184). Whenever imperative, though, autologous (left renal vein, jugular vein, splenic vein, saphena magna, etc) or allografts can also be used with increased risk of morbidity in the former case related to the devascularized area where the graft has been harvested from(186, 189, 190, 192-194). These observations furthermore raise the necessity for the extent of venous involvement to be redefined and characterized from a biological standpoint of view, not only technically. Only recently it has been recognized in a consensus statement that other factors than the vascular anatomical involvement should be considered to define more advanced cancer stage, such as the preoperative levels of Ca19-9 (162). Some recent papers confirm the value of the preoperative serum level of Ca19-9 as a predictor for worse survival after curatively intended pancreatic resection for PC (175, 179, 195). Also, the patients' general health needs to be considered, as it does have an impact on their chance for survival(162, 175).

Regarding what type of venous resection and reconstruction (direct suture, sleeve, patch, segmental resection, grafts) should be preferred, carrying the least risk for postoperative morbidity while being mostly oncologically safe, there is no sufficient information in the literature (161, 179). Small series are mostly focusing on the safety of interposition grafts and showing discrepant results, while primary suture and segmental resections are less commonly

compared (179). Only one paper from a large pancreatic center points out that the type of venous resection and reconstruction (as per ISGPS) does not have impact on the overall survival (164). On the other hand, the patients' general health, as described by a higher American Society of Anaesthesiologists (ASA) score (≥3) has been shown to be an independent prognostic factor for worse survival after PD, implying that better patient selection is crucial (164, 175). Which patients with venous involvement benefit from resection and how to select the better candidates for upfront surgery or neoadjuvant treatment is not entirely certain. Further studies are needed to discriminate the biologically advanced versus the technically "unfortunate" and tricky PC with venous involvement.

2.2.2.2. Arterial resections during pancreatectomy

While vein resections are considered standard approach in high-volume pancreatic centers, there is an ongoing debate regarding the indications for arterial resections in case of LAPC. Previous studies and a meta-analysis, all coming from small series, show that arterial resections are feasible, but associated with higher postoperative morbidity and mortality, while having worse long-term outcome (173, 196). The reported morbidity is about 53.6% (compared for 20-40% for standard resections), while the postoperative mortality – 11.8% (versus 2-3% for standard resection) (196). It is clear, though, that pancreatectomy with arterial resection provides survival advantage as compared to palliation (3-year survival of 8.3% (173, 196).

All these previous reports on arterial resections during pancreatectomy come from a period where potent oncologic regimens were lacking. With the introduction of FOLFIRINOX for metastatic and locally advanced pancreatic cancer(197), the prognosis of patients with LAPC have also changed and hence the interest for more aggressive surgery. For the past year, there is a storming amount of publications on the utility of arterial resections reporting much better survival (including 5-year survival of 12-23.4%) than older reports and also better perioperative outcome(147, 148, 176, 198). Our institution reported so far, the best perioperative outcomes, similar to these after standard resections – probably be due to the more careful patient selection, which proves to be a crucial factor(148). In the setting of NAT, though, arterial resections are less often necessary than expected. On the other hand, extensive dissection and clearance of all perineural and lymphatic tissue to the origin of the arteries from abdominal aorta (the so called "triangle operation") is essential(199). When arterial

resections are necessary, usually total pancreatectomy is performed to minimize the risks of pancreatic-fistula associated morbidity in the area of high-debit vessel reconstructed (Figure 1).

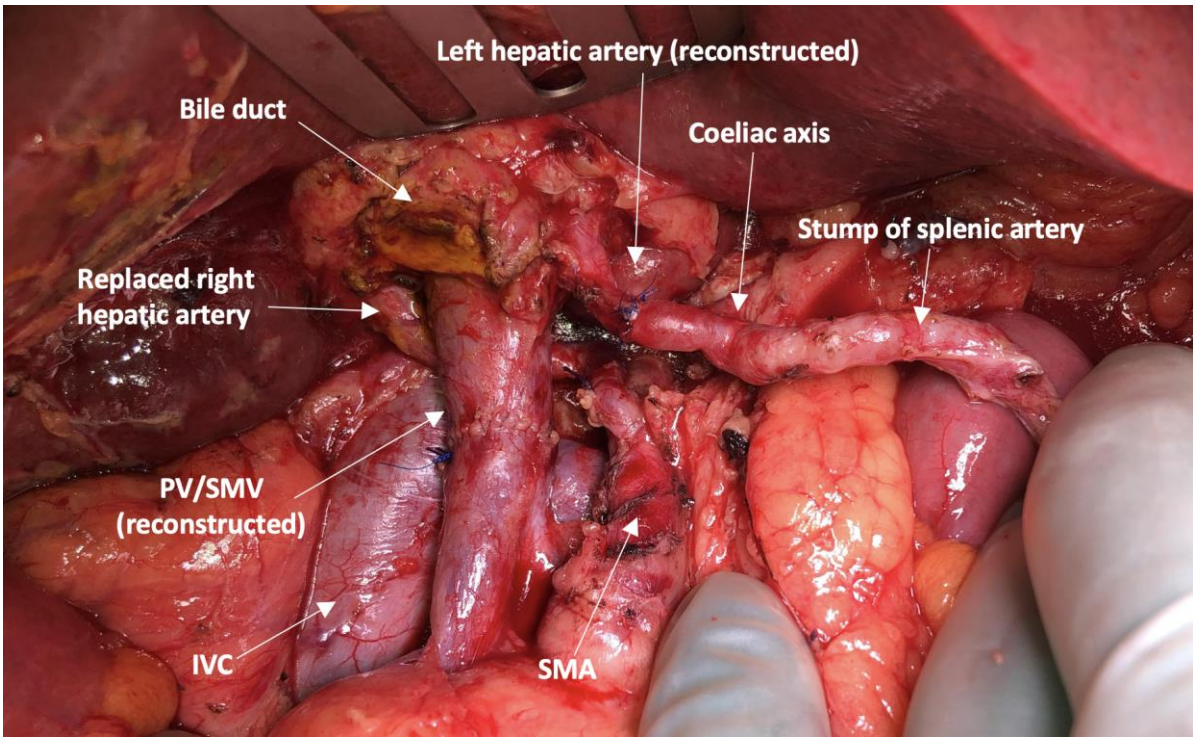


Figure 1. Standard surgical resection with combined arterial-venous resection and total pancreatectomy done for LAPC. Reconstructed with primary venous anastomosis after segmental resection of PV/SMV. Longer stump of the splenic artery is preserved until the end of the operation, to reassure an extra conduit for reconstruction is present in case problems with the arterial reconstruction occur peroperatively (*own archive*).

2.3. Medical oncologic therapy

As discussed previously, due to its biologic entities, pancreatic cancer is highly resistant to standard oncologic chemo- and radiotherapy. Therefore, these modalities alone or in combination with each other practically never lead to cure, but can only prolong the expected survival time.

Chemotherapy gives only a marginal survival advantage. The generally applicable chemotherapeutic regimens are based on gemcitabine or 5-FU, alone or in combination.

Lately, there has been much more focus on combination therapies rather than single-drug administration, as monotherapy. A recent Cochrane review points that combination therapies based on gemcitabine (together with nab-paclitaxel, platinum, etc), do show survival advantage for patients with advanced pancreatic cancer on the price of increasing the side effects (200, 201). The same seems not to be true for 5-FU based treatment (200). What gemcitabine can achieve in case of locally advanced disease is a median survival of 6-13 months (202).

Radiotherapy alone has hardly any effect on PC survival. Whether combination with chemotherapy could give survival advantage, the results are inconsistent. In the adjuvant setting, no advantage has been observed in the ESPAC-1 trial, and therefore not recommended(203). As part of the multimodality treatment of advanced non-resectable disease, there is a discrepancy in the applicability in the US in and Europe, the latter being more reluctant to apply it widely(168-170, 204, 205). There is increasing interest in the use of stereotactic body radiotherapy (SBRT) together with chemotherapy, for locally advanced disease, but larger prospective trials are needed to confirm its benefits(206-208).

2.3.1. FOLFIRINOX

FOLFIRINOX represents a combination of four cytotoxic drugs – oxaliplatin (85 mg/m^2), irinotecan (180 mg/m^2), leucovorin (400mg/m^2), and fluorouracil (400 mg/m^2 bolus plus 2400 mg/m^2 per 46-hour infusion), given every second week. After FOLFIRINOX came into the spotlight the odds for better survival even for the patients with LAPC and metastatic PC improved. Compared to standard gemcitabine-based treatment, FOLFIRINOX gives a substantial survival advantage of 24.2 months, i.e. similar to the survival reported for primary resectable pancreatic cancer(17, 197). FOLFIRINOX proved to be superior to gemcitabine even in the adjuvant setting and almost doubled the median disease-free and overall survival from 12.8 to 21.6 months and from 35.0 to 54.4 months, respectively (209). The effectiveness of FOLFIRINOX has been confirmed in a couple of meta-analyses (202, 210). Thus, the interest to FOLFIRINOX has grown as an induction therapy with the possibility for later surgical resection.

The effectiveness of FOLFIRINOX is accompanied inevitable by a quite extensive toxicity profile, which makes it, unfortunately inapplicable in the majority of patients(197, 202). A

pooled analysis of reported data revealed that grade 3 and 4 adverse events are being reported about 60 per 100 patients (202). A modified regimen, without a bolus dose, proved to be just as effective, but with improved toxicity profile(211, 212). Dose reductions are quite often necessary to deal with the adverse events and even in the initial report by Conroy *et al*, the median dose that the patients received was 80% of the planned dose(197). Dose reductions by 80% seem to keep the effectiveness of the drug combination(202, 211). Whether the regimen can be modified further to increase its applicability without jeopardizing its effect, is unclear.

Other combination therapies have started to emerge, such as gemcitabine – nab-paclitaxel, having better effectiveness than gemcitabine, but significantly improved safety profile (200, 201, 213, 214). Whether these would have an unequivocal role in the neoadjuvant setting still needs to be evaluated.

2.3.2. Neoadjuvant therapy and surgery for LAPC

Together with establishment of more effective oncologic therapy for LAPC, the interest for more aggressive surgery has re-awakened. Blazer *et al* clearly demonstrated, that not only FOLFIRINOX gives clear survival advantage, but also treatment with modified FOLFIRINOX followed by surgical resection leads to substantial difference in prognosis – progression-free survival of 18 months compared to 8 months if no resection was performed (212). Also, after FOLFIRINOX, much higher proportion of patients can be resected compared to other chemotherapeutic regimens – 61% versus 46%, also with clear survival advantage compared to non-resected patients – median and 3-year survival of 16 and 8.5 months and 28.1% versus and 2.4%, respectively (215). Reports keep being accumulated confirming that resection after induction oncologic therapy can improve dramatically the prognosis of patients with LAPC(16, 205, 216-218). A meta-analysis, including 13 studies and 253 patients treated with FOLFIRINOX, showed a secondary resection rate of 43% and an R0 resection in 64% of patients with borderline and 23% with LAPC(210).

Even with the intention of more aggressive surgery, the key role of NAT remains in providing significant survival advantage to patients with LAPC (217). The survival of resected patients with LAPC without upfront NAT was reported to be worse(217). The purpose of NAT is not only to incur cytolytic effect on the tumor cells, but also to select the patients with more favorable tumor biology – without underlying aggressive metastatic disease or clear local

progression, so that they would benefit from local treatment strategy that surgery is. NAT leads to increased detection of smaller tumors and lymph-node negative disease on final histology and decreases the rate of local recurrence, thus potentially enhancing the benefit of surgical resection (215, 217-220). Standard pathological assessment after NAT in reporting resection margins, though, cannot be applied in the same way as with upfront surgery and it seems meaningless due to the different tissue architecture and distance among tumor cells(221). Only almost-complete tumor regression, with <5% viable tumor cells after NAT is associated with better disease-free, but not overall survival(205, 222).

There is currently no consensus what the duration of NAT should be. The length of the reported regimen duration usually varies between 2 to 6 months. Theoretically it should be long enough to observe and select the tumors with "better" biology, but not too long to develop adaptation and select resistant to the agents tumor cell clones. How long the optimal time is, still needs to be evaluated. One report suggests that duration of chemotherapy more than 6 cycles is associated with improved 1-,2-, and 3-year survival(205).

2.3.3. Prediction of response to neoadjuvant therapy

To distinguish which patients have responded to NAT and would benefit from an aggressive surgical approach is a crucial but also a very difficult task. The most reliable criteria are the histologic verification of tumor clearance. However, this information is only available after surgical resection and thus is unable to guide clinical decision-making which patient to resect. Other criteria need to be applied.

2.3.3.1. Radiologic re-evaluation

The standard approach to assess resectability is by radiologic description of the relation of the tumor to the peripancreatic vessels. There are no clear criteria for good response to NAT as the standard radiologic response evaluation criteria in solid tumors (RECIST) are not very useful in PC(218, 223, 224). Due to the tumor's extensive fibrotic content that does not disappear with oncologic therapy, down-sizing of the tumor is unusual, while stable disease is what is most frequently observed(16, 218, 224). Viable tumor can hardly be distinguished from fibrosis even microscopically on histology, and the task is basically unachievable when

reading the post-treatment radiology (218). The radiologic examination after NAT may be helpful to identify patient with metastatic spread of disease or clear local progression. Whether the phenomenon of pseudo-progression, as a sign of good treatment response, occurs even in PC is unknown. Radiologic imaging is, though, a very poor tool identifying the exact tumor size and the degree of vascular extension as well as the amount of vital tumor persisting. Ferrone *et al* reported that despite that post-treatment imaging still pointed to irresectability in 48% of the patients with LAPC (pre-treatment – 65%), R0 resection was achieved in 92% of the patients when surgery was attempted. Preoperative radiologic re-evaluation is unable to detect tumor regression and predict resectability, and therefore not able to guide clinical decisions to proceed to resection (218, 225). The current consensus is that patients not exhibiting progress during NAT are worth surgical exploration and an attempt for resection.
It still needs to be standardized what the standard NAT regimens of choice should be in case FOLFIRINOX is inapplicable, what the optimal duration of therapy should be, and how aggressive the surgical approach should be in relation to how effective the oncologic therapy is predicted to be.

2.3.3.2. CA19-9

The radiologic appearance of PC after NAT defines the technical aspects of the tumor extent and helps prepare the surgical strategy, but does not truly mirror tumor biology. A marker that has been long used for risk stratification of patients with PC and having a good predictive value for survival is serum level of carbohydrate antigen 19-9 (CA19-9)(226, 227). Different cut-off values above the normal (37 U/ml) have been evaluated (100 to 500) and most often found to be related to shorter survival(98, 162, 164, 175, 179, 195, 205, 215, 217, 227). Usually, levels below 100 U/ml suggest resectable disease, while levels > 100 are more often indicative of unresectable or metastatic disease (226).

What the cut-off is most predictive for outcome in resectable patients is debatable, though. What might make the evaluation of a cut-off difficult is the false-positive higher elevation of CA19-9 in case of non-decompressed obstructive jaundice. Normal preoperative CA19-9 points to a significantly better survival compared to elevated levels. Normalized CA19-9 after primary surgical resection and adjuvant treatment or a drop of at least 30-50% was also

related to significantly improved survival while the opposite predicted early recurrence (226, 228).

CA9-9 is able to predict survival in BRPC and LAPC, too. Lower levels or a drop of CA19-9 (between 20 to 90%) during oncologic treatment (chemo- or chemo-radiotherapy) is indicative of good response and improved survival (229-235). In case NAT is followed by an attempt for resection, preoperative CA9-9 is again indicative for outcome. Higher values > 100 U/ml were associated with higher risk for both local and distant recurrence and impaired survival (216, 236). Decrease of CA19-9 (more than 30 to 50%) or normalization were both predictors of better survival(205, 224, 237). CA19-9 has also been correlated to pathologic response –decrease of >90% correlated with complete response of 29% versus 0% (237). CA19-9 is, though, still not generally considered a factor to guide therapy switch or preclude exploration after NAT. What should the exact cut-off be to change the therapeutic algorithm and whether there are values at which surgical resection does not bring any benefit is uncertain.

2.3.3.3. Para-aortic lymph nodes

Positive metastatic para-aortic lymph nodes (PALN) are considered M1 disease according to the TNM classification. There is a quite uniform consensus that surgery should not be considered in case of M1 disease with location in other organs (for instance liver and peritoneum). The debate is, however, still ongoing what the strategy should be in case of positive PALN, despite that there are extremely few 3- and almost no 5-year survivors with PALN+ (238-253). Disregarding the TNM classification, there is a discussion whether the prognosis of PALN+ patients resembles more that of M1 patients with spread to other organs or N2 disease (238, 254) and no consensus has been reached (255, 256). There is no clear-set anatomic background how the spread to PALN occurs – via direct posterior infiltration by the tumor, lymphogenic, via haematogenic pathway or via multiple routes (243, 257, 258). Whether PALN involvement is more common in pancreatic-head than in pancreatic-tail tumors is also uncertain as the data in literature are contradicting (243, 259).

It is likely that the incidence of metastatic PALN (10-24%) is underreported(243, 246, 249, 260, 261). Komo *et al* discovered almost double as many micrometastases on specific immunohistochemistry staining that was not captured on standard hematoxylin-eosin (HE)

staining (244). The patients with micrometastases to PALN, however, had the same dismal prognosis as in patients with HE positive PALN.

PALN are generally not being sampled routinely during surgery for primary resectable PC. Data in literature on survival in case of PALN+ comes mostly from series of resected patients with PALN+, but are very scarce on non-surgical series (252, 262), when PALN+ have been considered a contraindication for primary resection. Many studies, surprisingly, do not report at all 3-and 5-year survival and one could speculate that this is due to the much unfavorable long-term outcome. The 3-year survival is reported to be between 0% (241, 245, 247, 252, 253) and 10.6% (238-240, 242, 243, 246, 248, 249). Only one study reports a 3-year survival of 16.7% (260). The reported 5-year survival is most often 0%, with only few reporting a survival of 6.8% or predicted survival about that percentage (238, 244, 251, 260). Interesting, the reported better survival rates usually comes from more recent studies, where probably even more potent medical oncologic treatment was available. The prognosis of PALN+ resected patients was shown to improve after adjuvant chemotherapy (244, 260). On the other hand, in a small series of patients, where PALN+ were contraindication for resection and were treated with chemotherapy, the reported survival was surprisingly favorable – (263).

There is hardly any data as to what the approach to patients with BR-LAPC should be after NAT in case of PALN+. The problem, however, seems to be even more pending and relevant than in primary resectable PC, since few papers are reporting higher incidence of PALN+ with stage T3/4 (247, 259, 260) and the presence of venous and arterial invasion, and even worse survival (92, 242, 253). The issue has hardly been addressed and there is no strategy how to deal with PALN+ in LAPC patients. NAT has not been shown to improve the prognosis of resected PALN+ patients. Whether this may indicate failure to respond to NAT and possibly a contraindication for resection has not been investigated either.

The reluctance to consider a different approach in case of PALN+ depends partially on the fact that lymphadenectomy is easy to perform without having significant impact on morbidity and partially because metastatic lymph nodes are very difficult to detect on preoperative imaging (264). Imai reported a series of patients with PALN+ on final histology where none were visible on preoperative CT, MRI or FDG-PET (264). Thus, when the decision for surgery has been already taken, it is more difficult to draw back, even if frozen section is

considered before continuing with resection. Schwarz *et al* found that frozen section can still miss to detect about 30 % of PALN+, confirmed only on final histology (249).

To predict the presence of PALN+ might help guide better therapeutic decision-making and the best timing of surgery, for instance after NAT, even in primary resectable cases. Other biomarkers than standard imaging might need to be sought. A correlation has been observed between elevated Ca 19-9 and PALN+. In resectable patients elevated CA19-9 was correspondent to higher risk for PALN+ detection(253). Asaoka *et al* observed better survival in resected PALN+ patients with Ca19-9<360 U/ml (239). On the other hand, PALN+ have been associated with higher postoperative values of Ca 19-9 and early recurrence (262). How exactly CA19-9 should be used to predict the presence of PALN+ or whether these are two independent prognostic factors for progressive PC has not been systematically addressed.

PALN status is traditionally regarded as a postoperative prognostic marker. However, being able to predict possible metastases and sample these lymph nodes, for instance either via EUS or by mini-invasive surgical technique, or even by open resection, might allow PALN to be used as a preoperative marker, used to navigate the tactical decision towards resection or other treatment strategy.

3. Aims

The aims posed by this thesis meant to addressed two substantial pending issues in PC. The first one is the concern whether the currently used classifications for local tumor involvement are able to reflect the biologic tumor behavior and whether the technical stand-points are still predictable of outcome. The second issue is whether different treatment opportunities for are at all currently conceivable, by manipulating the host's biologic features as to achieve anti-tumoral effect.

Thus, the aims were as follows:

1. To rule out what the safest technique for venous resection during pancreatectomy is and to identify features associated with inferior survival - whether they are technical or biologic in nature and thus when VR should be performed.

2. To investigate whether locally advanced pancreatic cancer is a "surgical" disease– whether resection brings survival benefit in the multimodality setting and in what scenarios it should be attempted.

3. To evaluate whether potentially available preoperatively markers reflecting tumor biology (Ca19-9, mGPS, para-aortic lymph node involvement) can better reflect tumor behavior than standard classifications in patients with localized disease undergoing surgery.

4. To investigate whether current classifications for local tumor extension are related to patients' cancer prognosis – could they adequately predict the odds for survival with the currently applied treatment strategies.

5. To investigate whether TILs from PC could be successfully isolated and expanded *ex vivo* as to be used potentially for therapy.

6. To determine the TILs phenotype – whether they have recognized TAAs and show reactivity against autologous tumor cells in culture.

4. Methods

Study I, II, and III were retrospective observational cohort studies. Ethical permission was obtained from the ethical committee board in Stockholm, Sweden. The data was retrieved from a prospectively gathered institutional database. The studies were conducted using the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for observational studies(265). The study period in study I and III encompassed patients operated between January 2018 up to January 2019. The study period for study III was 2007 to 2017 as the data was obtained from MDT conference registry where the diagnosis was confirmed and a decision of NAT was taken.

Standard methods for descriptive statistics were used. Categorical variables were presented as frequencies (*n*) and percentages (%). Continuous variables were presented by median and range. Chi-square test was used to compare categorical data. *t*-test was used for variable comparison for normally distributed continuous data. Sex- and age adjusted univariable or multivariable logistic regression with odds ratios (ORs) and 95 % confidence intervals (95 % CI) were used for analyzing risk factors for surgical complications and survival for variables that showed a significant association in the comparative tests in study II. Statistical significance was set at $p < 0.05$.

Survival in study I and III was defined as the time elapsing from surgery until 5 years or death, whichever came first. For study II, the starting point of the survival estimate was defined as time of diagnosis as only one of the study cohorts made it to surgery. The starting time point was chosen, since it is the most reasonable one from the patients' perspective. Kaplan-Meier estimates were used for survival analysis and group comparison was performed by log rank test in study I and III. For study II, the association between resection and mortality was quantified using the Cox proportional hazard model further adjusting for age, gender, type of NAT, and CA19-9. The quantitative predictor CA19-9 was modeled using restricted cubic splines with 3 knots at fixed percentiles of the distribution. The possible heterogeneous effect of resection on mortality was examined along the range of CA19-9. The product terms between the two splines of Ca19-9 and resection were included in the multivariable Cox model further adjusted for age, gender, and type of chemo. A p-value for interaction was obtained by testing the coefficients of the two splines equal to zero using a Wald-type test. The mortality hazard ratio with 95% confidence for resection was visualized

as function of Ca19-9. A complete-case analysis was done to deal with missing data in the predictors.

Two more models were applied in study II, to eliminate the possible confounding effect of the immortal time bias on surgery being able to influence survival. In the first model, the survival was defined, instead of starting from time-point of diagnosis, starting from date of surgery for the resected ones or end date of NAT for the non-resected ones. In the second model, date of diagnosis was considered as a starting point of survival estimation, while resection was considered as a time-dependent indicator variable, which takes the value of one or zero at any time point.

In study IV, ethical permission was again obtained from regional ethical review board in Stockholm, Sweden. The analyses were performed on tumor tissues obtained from a cohort of operated patients by biopsy or tumor excision. Initial cultures were done on 24-well-plated using Cellgro GMP serum-free medium and 10% human AB serum, supplied with IL-2, IL-15, and IL-21, together with penicillin, streptomycin, and amphotericin B. After 7 days, the cultures were split to additional plates and after 10 days were expanded using OKT-3 and 55Gy-irradiated allogenic feeder cells in ratio 1:10, and with the same cytokine cocktail. If then activity to TAA was detected by IFN-gamma production, TILs were expanded in GRex flasks. For phenotyping, TILs were stained with respective antibodies for activation and exhaustion markers and analyzed by flow cytometry. TCR Vβ frequency analysis was done by staining via the TCR Vβ Repertoire Kit and analyzed by flow cytometry. TCR CDR3 analysis was performed by PCR. IFN-gamma ELISA was performed after stimulation with a selected peptide mix. For each peptide mix, blocking was performed using an MHC-class I or class II mAb (w6/32 and HLA-DR, respectively) to determine whether adequate antigen-presentation took place. Cytotoxic T-cell responses were measured by Chromium 51 release essay. A serial dilution was performed to achieve ration to tumor cells of 25:1 to 1.56:1. Tumor target cells were labeled by Na_2CrO_4 and after culturing the Cr51 radioactivity of the supernatant was measured.

5. Results

The main finding in each study are presented in this chapter. More detailed description of the results is available in the respective original paper. Some extra data is presented that helps clarify the overall conceptual results, but did not find place in the four manuscripts.

5.1. Study I

Altogether, 318 venous resections with pancreatectomy were performed, in 290 patients isolated VR, without combined arterial resection, comprising the study group. Most of the resections were done for PDAC (n=188). Out of them, 131 patients had primary resectable tumors, 40 – BRPC, and 17- LAPC. NAT was administered to only 1 patient with resectable tumor (0.8%) and 16 patients with LAPC (94%) with LAPC ($p<0.0001$). The adjuvant chemotherapy, though, was similar ($p=0.69$).

Most of the resections performed were PD (n=202, 70%) and TP (n=75, 26%), in 62 patients (21%), combined with multi-organ resections. Most often resection of both PV / SMV was necessary, including the spleno-mesenteric confluence (53%). Primary reconstruction with a direct single anastomosis could be performed in 280 patients (97%), as the preferred method shifted from wedge resection (type 1, ISGPS) in the beginning of the period to segmental resection later on (type 3), as up to 7 cm long segments could be thus be resected.

Analysis of the perioperative complications revealed that morbidity and mortality rates were similar to what has been reported after standard pancreatic resections – 56% surgical complications and 4.1% 90-day mortality (Table 1). The predominant surgical complication was DGE (38%) as in 60% of cases it was primary. Severe surgical complications (Clavien-Dindo grade ≥3b), were encountered in 11% of patients, another 13 % had complications requiring intervention (grade 3a). The general reoperation rate was 8%, but the rate due to troubles with the vascular reconstruction itself was only 1.4% (n=4). The rate of vein thrombosis was 4.5%, while other thromboembolic events occurred in 8.3%. Distal pancreatectomy was associated higher risk for thrombosis than PD and TP (38% versus 3% and 4%, respectively, $p<0.001$), but pancreatic fistula was not (10.5% vs 4.4%, $p=0.23$). The type of venous reconstruction generally did not have an impact on the development of

thrombosis (4.1 and 4.3 % for type 1 and 3, respectively none in type 2 and 4). On univariate analysis obesity, combined PV/SMV resection, and multi-organ resection (gastrectomy in particular) were associated with higher risk for developing severe surgical complications, but this was not confirmed on multivariate analysis (Table 2).
The survival among the three most common types of periampullary cancer, PDAC, IPMN cancer and distal cholangiocarcinoma, was not statistically significant: median, 1-, 3-, and 5-year survival was 18, 16, 18 months, respectively (p=0.8, Figure 2). For the PDAC patients, the survival was similar to that patients operated for head and neck PDAC with standard operation (no VR) during the same study period. The survival among patients undergoing surgery with VR for resectable PDAC, BRPC, and LAPC was not statistically significant either: median survival of 18, 14, and 23 months (p=0.7, Figure 3). Outside the spectrum of the manuscript, comparison between the survival of patients with isolated VR and combined arterial-venous resection was performed and found not to be statistically significant either. The median survival time was 22 and 17 months (p=0.6, Figure 4). There was no difference in survival either according to the four types of venous reconstruction: median survival for type 1, 2, 3, and 4 was 21, 21.5, 18, and 16 months (p=0.7, Figure 5, not included in the manuscript).

On uni- and multivariate analysis considering factors that might be associated with shorter survival than the median for standard operation without VR (that was 19 months), only elevated Ca 19-9 and ASA score ≥ 3 or above were associated with impaired survival (Table 3). M1 disease (in 85% due to metastases to PALN), showed the strongest association with impaired survival on univariate analysis (OR with 95% CI 3.17(1.31-7.66)), but failed to reach statistical significance on multivariate analysis.

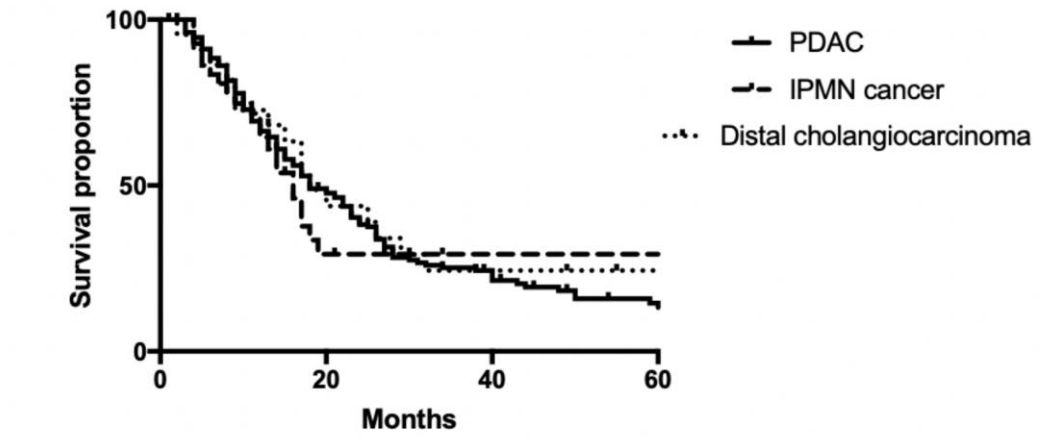
Table 1. Postoperative outcome

Overall complications, n (%*)	180 (62)
Surgical complications, n (%)	163 (56)
DGE	111 (38)
POPF**	19 (9)
GI bleeding	23 (8)
Intraabdominal bleeding	13 (4)
Anastomotic leak	10 (3)
Abscess	26 (9)
Vein thrombosis	14 (4.8)
Medical complications, n (%)	62 (21)
Thromboembolic	24 (8.3)
Other	49 (17)
Reoperations	23 (8)
Due to problems with venous resection/reconstruction	4 (1.4)
Clavien-Dindo classification	
1-2	112 (39)
3a	37 (13)
≥ 3b	31 (11)
30-day mortality***	8 (2.8)
90-day mortality***	12 (4.1)
Length of hospital stay, median (range)	10 (0-112)

Table 2: Uni- and multivariate analysis of possible contributing factors to severe postoperative complications (Clavien-Dindo ≥3b).

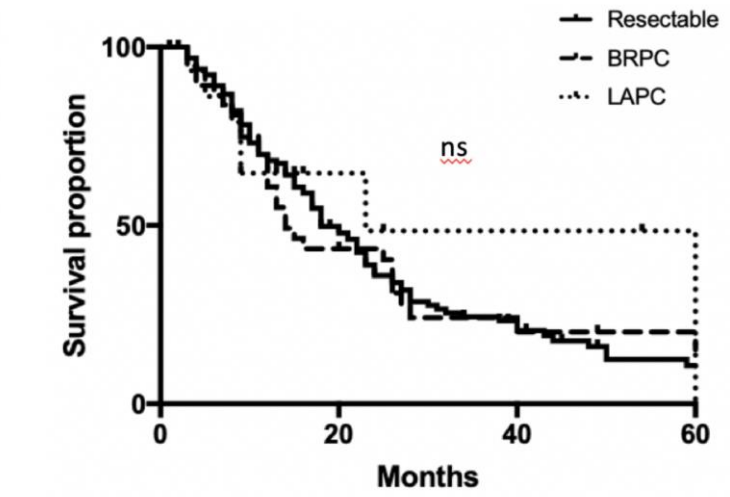
Factor	Clavien-Dindo < 3b		Clavien-Dindo ≥ 3b		p value
	n	%	n	%	
Number of patients	149			31	na
Median Age	68	67 - 70; 95% CI	68	62 - 70; 95% CI	0.26
Sex (female)	70/149	47 %	14/31	45 %	0.85
ASA ≥3	43/149	29 %	10/31	32 %	0.70
Underweight	**4/149**	**2.7 %**	**1/31**	**3.2 %**	**0.06**
Normal weight	**79/149**	**53 %**	**12/31**	**39 %**	
Overweight	**49/149**	**33 %**	**13/31**	**42 %**	
Obesity	**17/149**	**11.4 %**	**5/31**	**16 %**	
Cardiologic co-morbidity	73/149	49 %	13/31	42 %	0.47
Respiratory co-morbidity	13/149	8.7 %	2/31	6.5 %	0.68
PV resection	25/149	17 %	3/31	9.7%	0.32
SMV/PV resection	**75/149**	**50 %**	**23/31**	**74 %**	**0.01**
SMV resection	44/149	30%	5/31	16%	0.13
IVC resection	3/149	2.0 %	0	0	0.43
Vein Resection Type					
1	54	36.3 %	8	26%	0.27
2	3	2%	1	10 %	0.68
3	90	60.4 %	22	71%	0.27
4	2	1.3 %	0	0	0.52
Length of resected vein (cm)	2.5	2.0-3.0, 95% CI	2.5	2.0-3.0, 95% CI	0.34
Multi-organ resection	**33/149**	**22 %**	**14/31**	**45 %**	**0.008**
Colonic resection	9/149	6 %	4/31	13 %	0.18
Gastric resection	**13/149**	**9 %**	**7/31**	**22 %**	**0.03**
Median operation time (min)	421	(404-432, 95% CI)	387	(344-429, 95% CI)	0.08
Neoadjuvant CHT	21/148	14 %	4/31	13 %	0.86
Radiotherapy	7/149	5 %	3/31	9.7 %	0.27
Resectable	96/144	67 %	20/30	67 %	1.00
BRPC	36/144	25 %	9/30	30 %	0.65
LAPC	12/144	8.3 %	1/30	3.3 %	0.47
PDAC	**101/149**	**68 %**	**14/31**	**45 %**	**0.01**
IPMN cancer	16/149	11 %	4/31	13 %	0.73
Distal cholangiocarcinoma	14/149	9.4 %	2/31	6.5 %	0.60

Factor	OR 95% CI (Univariate)	p value	OR 95% CI (Multivariate)	p value
Overweight	1.73 (0.76-4.01)	0.18		
Obesity	**3.40 (1.01-11.41)**	**0.04**	2.80 (0.79-9.88)	0.10
SMV/PV resection	**3.01 (1.28-7.04)**	**0.01**	2.70 (0.81-9.03)	0.10
Multiorgan resection	**3.52 (1.62-7.66)**	**0.0015**		
Gastric resection	**2.95 (1.14-7.66)**	**0.02**	1.60 (0.35-7.17)	0.53



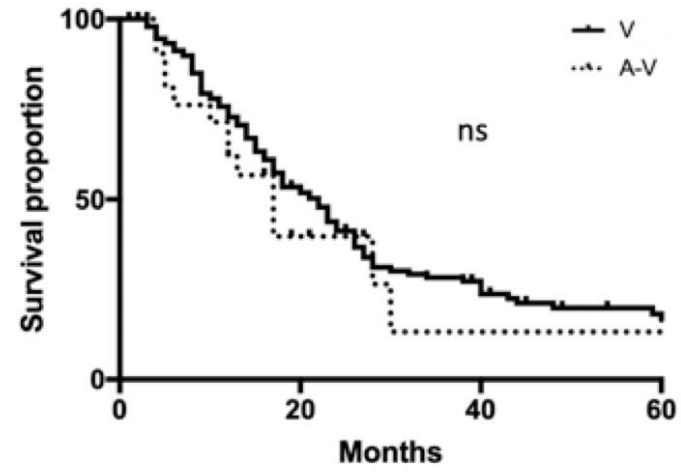
N at risk				
PDAC	183	75	25	12
IPMN cancer	37	8	3	3
Distal	22	10	5	3

Figure 2: Kaplan-Meier survival analysis of resected with VR PDAC, IPMN cancer, and distal cholangiocarcinoma. Median, 1-, 3-, and 5-year survival was 18, 16, 18 months, and 66%, 65%, 73%; 25%, 29%, 24%; and 15%, 29%, 24%, respectively (p=0.8289).



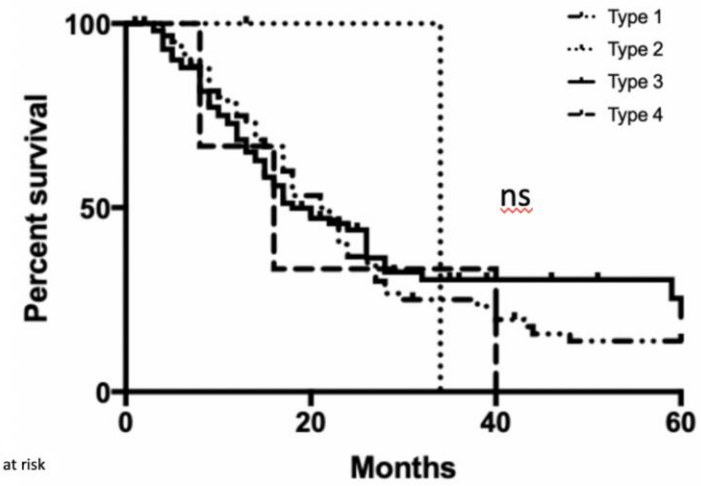
N at risk				
R	129	56	17	7
BRPC	37	15	6	5
LAPC	17	5	3	2

Figure 3: Kaplan-Meier survival analysis of resected with VR patients with resectable, BRPC, and LAPC undergoing pancreatectomy with venous resection. The median, 1-, 3-, and 5-year survival of 18, 14, and 23 months, and 68%, 61%, 65%; 24%, 24%, 48%; and 11%, 20%, 48%, respectively (p=0.7361).



N at risk				
V	151	68	23	12
A-V	22	7	2	2

Figure 4: Kaplan-Meier survival analysis of resected patients with isolated VR and combined arterial-venous resection *(not included in manuscript)*. The median, 1-, 3-, and 5-year survival were 22 and 17 months; 73% and 62%; 28% and 13%; 17% and 13%, respectively (p=0.6200, ns).



N at risk				
Type 1	60	35	13	7
Type 2	2	12	1	1
Type 3	105	37	10	6
Type 4	3	2	1	1

Figure 5: Kaplan-Meier survival analysis of resected patients with VR according to the four types of venous reconstruction according to ISGPS *(not included in manuscript)*. The median survival for type 1, 2, 3, and 4 was 21, 21.5, 18, and 16 months; 1-, 3-, and 5-year survival of 70%, 50%, 64%, and 67%; 21%, 0, 31%, and 33%; and 11%, 0, 20% and 0, respectively (p=0.7)

Table 3: Uni- and multivariate analysis associated with shorter survival than the median for standard pancreatic resection of 19 months.

Factor	Survival ≥ 19 months		Survival <19 months		P value
	N	%	n	%	
Number of patients	76			108	na
Median Age	68	65-69; 95% CI	68	67-71; 95% CI	0.7
Sex (female)	36/76	47 %	58/108	54 %	0.39
ASA ≥3	**10/76**	**13 %**	**33/108**	**30 %**	**0.006**
Underweight	0/76	0	4/108	3.7 %	0.83
Normal weight	44/76	57.9 %	57/108	53 %	
Overweight	27/76	35.5 %	37/108	34 %	
Obesity	5/76	6.6 %	10/108	9.3 %	
PV resection	12/76	16 %	14/108	13 %	0.59
SMV	26/76	34%	33/108	30%	0.60
SMV/PV resection	37/76	49 %	59/108	55 %	0.43
IVC resection	1/76	1.3 %	1/108	1 %	0.80
Vein Resection Type					
1	34/76	45 %	33/108	30 %	0.07
2	1/76	1 %	3/108	3 %	0.50
3	40/76	53 %	70/108	65 %	0.09
4	1/76	1 %	2/108	2 %	0.78
Multiorgan resection	9/76	12 %	18/108	17 %	0.36
BRPC	15/76	20 %	23/108	21 %	0.76
LAPC	4/76	5 %	13/108	12 %	0.11
Neoadjuvant CHT	10/76	13 %	16/108	15%	0.75
Adjuvant CHT	43/63	68 %	45/85	52 %	0.06
Radiotherapy	4/76	5 %	6/108	6 %	0.93
Median CA19-9	**67**	**47-116; 95% CI**	**308**	**150-514, 95%CI**	**0.0002**
Elevated CA19-9>200	**23/73**	**32 %**	**57/104**	**55 %**	**0.002**
LNR≥0.1	64/76	84 %	94/108	87 %	0.59
LNR≥0.2	**27/76**	**36 %**	**65/108**	**60 %**	**0.001**
T1	1/76	1.3 %	1/108	1 %	0.80
T2	2/76	2.6 %	8/108	7 %	0.16
T3	72/76	95 %	99/108	92 %	0.42
T4	1/76	1.3 %	0/108	0	0.23
pN	67/76	88 %	100/108	91 %	0.30
L1	**61/76**	**80 %**	**95/104**	**93 %**	**0.03**
V1	**56/76**	**74 %**	**94/107**	**88 %**	**0.01**
Pn1	69/73	95 %	102/105	97 %	0.38
pM1	**7/76**	**9 %**	**26/108**	**24 %**	**0.01**

Cont. of Table 3:

Factor	OR 95% CI (Univariate)	p value	OR 95% CI (Multivariate)	p value
ASA≥3	**2.56 (1.14-5.75)**	**0.02**	**2.61 (1.06-6.41)**	**0.03**
Elevated CA19-9	1.92 (0.94-3.94)	0.07		
CA19-9≥200	**2.60 (1.38-4.90)**	**0.02**	**2.52 (1.27 -4.99)**	**0.007**
LNR>0.2	**2.66 (1.44-4.92)**	**0.001**	1.89 (0.93-3.81)	0.07
L1	**2.86 (1.16-7.04)**	**0.02**		
V1	**2.54 (1.16-5.58)**	**0.01**	1.97 (0.81-4.80)	0.13
pM1	**3.17 (1.31-7.66)**	**0.01**	2.07 (0.75-5.71)	0.15

5.2. Study II

Altogether, 233 patients were eligible for NAT, but only 72.1% (n=168) did receive the planned treatment. Of these, 156 had PDAC and the majority were LAPC (84.6%, n=132). FOLFIRINOX was administered to 34.6% of the patients. Dose reduction and drug-combination modification for any chosen NAT were necessary in 59.7% of the patients.

Surgical exploration was attempted in 48.7% (n=76) of cases, resulting in resection in 68.4% of these (n=52). In 80.8% vascular resection (VR and/or arterial) was needed and in 65.4% - multi-organ resection. The surgical morbidity and 90-day mortality were low – 48% (in 36% represented solely by primary DGE) and 6%, respectively. For the 77% of the patients (n=40) in whom follow-up data was available, the recurrence frequency and pattern was not different for patients with BRPC and LAPC – 70% versus 60%. All patients had distant metastases and only 29% and 17%, respectively, had also local recurrence.

As in study I, the survival among patients with BRPC and LAPC was not statistically different, neither for all patients (Figure 6a), nor for the surgically resected ones (Figure 6b). For the latter, the median survival was 31.9 and 21.8 months, respectively, and 1-, 3-, and 5-year survival was 81.8, 43.6, and 32.7 % versus, 89.6, 39.5, and 25.4 %, respectively (p=0.7).

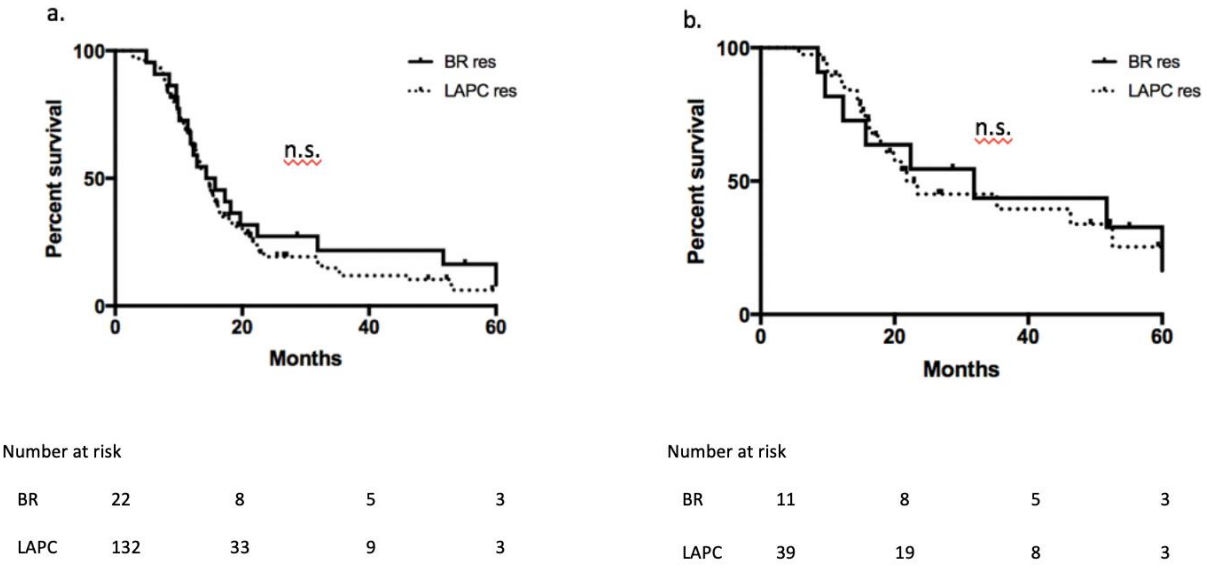
Resected patients had 73% lower mortality rate compared to non-resected ones (95% CI = 0.18, 0.42), with negligible impact after age and sex adjustment for 72% lower mortality rate (95% CI = 0.18, 0.43, Figure 7). After adjusting for preoperative Ca 19-9 and type of NAT, the association was slightly attenuated, but yet, mortality rate among resected patients was still 63% lower (95% CI = 0.22, 0.62).

Two more statistical models were applied to adjust for the possible effect of the immortal time bias. In the first model, resected patients had 73% lower mortality rate (95% CI: 0.17–0.43), an improved 1-year survival of 40% (resected: 60%, non-resected: 20%), and an improved median survival of 10.9 months (resected: 17.2 months, non-resected: 6.3 months. In the second model, again, resected patients had 69% lower mortality rate (95% CI, 0.20–0.49) and improved 1-year survival of 24% (resected: 82%, non-resected: 58%), and improved median survival of 8.9 months (resected: 21.9 months, non-resected: 13 months). A continuation of the series, after the study was closed for analysis (and therefore the data is not

part of the paper), compared patients undergoing exploration, without having signs of distant metastases – one group was resected and the other not, as there were no technical premises for safe reconstruction. The survival of the resected patients was significantly superior (Figure 8).

The 5-year actuarial survival of patients resected after FOLFIRINOX was 46.2% compared to 27.6% after other combination therapies. This difference was not significantly different. Even significant dose reductions of any form of NAT did not have a significant impact on survival whenever followed by surgical resection (Figure 9). For the group of patients who were not resected, though, dose reduction of FOLFIRINOX did not have significant impact on survival, while administering any other type of combination therapy, though, in reduced dose, was associated with inferior survival (Figure 9).

At last, the hazard ratio of mortality conferred by resection was investigated – whether it may vary according to different levels of Ca 19-9, adjusted for age, gender, and type of NAT. Although the risk for mortality increased with increasing levels of Ca19-9, surgical resection seemed to bring some survival benefit for all levels (Figure 10).



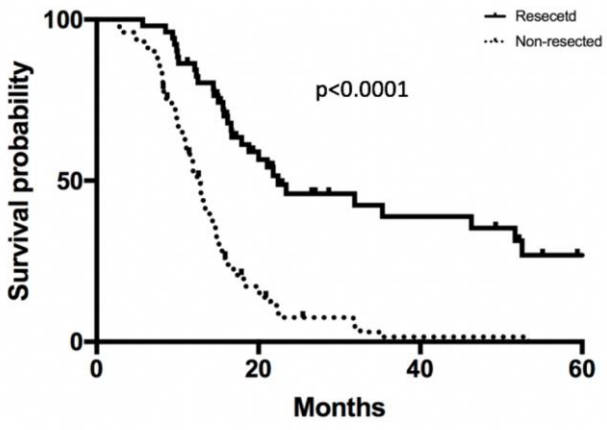
Number at risk

BR	22	8	5	3
LAPC	132	33	9	3

Number at risk

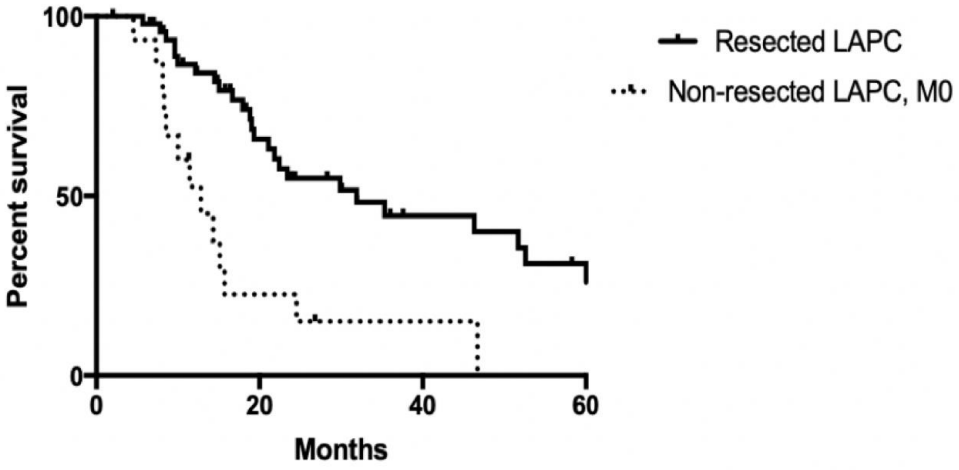
BR	11	8	5	3
LAPC	39	19	8	3

Figure 6: Kaplan-Meier survival analysis of patients with BRPC (solid line) and LAPC (dotted line) undergoing NAT: **a**. Overall survival; median survival of 15.0 versus 14.5 months respectively (p=0.4) and **b**. Survival after surgical resection; median survival of 31.9 versus 21.8 months (p=0.7).



Number at risk				
Resected	52	26	12	5
Non-resected	102	15	2	0

Figure 7: Kaplan-Meier survival probabilities for resected (solid line) and non-resected patients (dash line) – median survival of 22.4 versus 12.7 months; 1-, 3-, and 5-year survival of 86.4, 38.9, 26.9 % versus, 52.2, 1.5, 0 %, respectively (p<0001).



N at risk				
Resected	48	25	11	7
Non-resected	15	4	2	1

Figure 8: Kaplan-Meier survival analysis of patients with stable LAPC undergoing exploration, with no signs of M1. Resected (solid line) and non-resected patients (lack of technical possibility to reconstruct (dotted line). The median, 1-, 3-, and 5-year survival of the resected and non-resected patients was 32 and 13 months, 84% and 53%, 45% and 15%, and 26% and 0, respectively (p=0.0002).

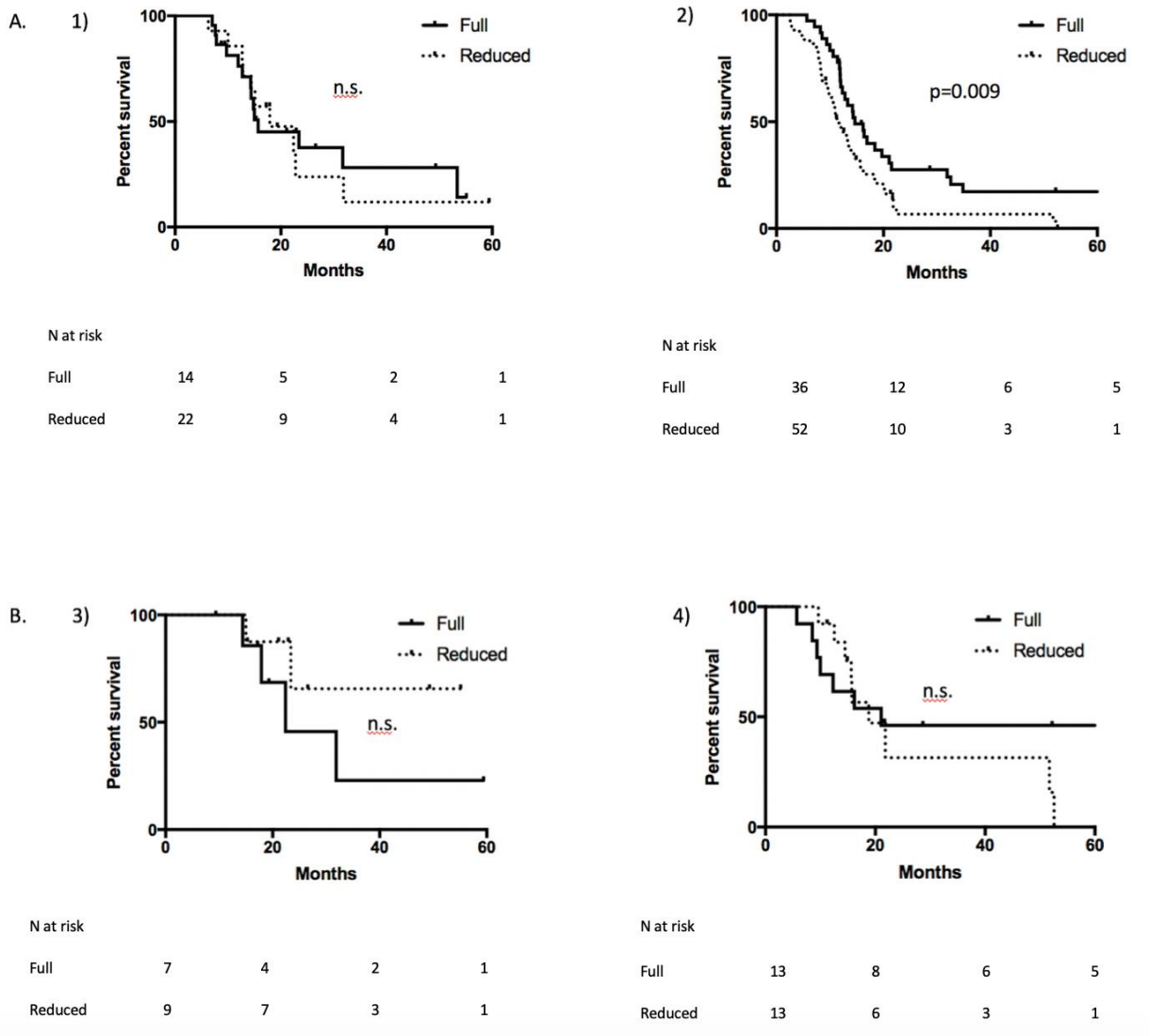


Figure 9: Survival after full and reduced dose chemotherapy in resected and non-resected patients: A. Overall survival after: 1) full dose and reduced dose FOLFIRINOX, median survival 17.9 versus 15.7 months (p=0.9) and 2) full and reduced dose other NAT: median survival 14.7 versus 11.5 months (p=0.009). B. Survival after resection after: 3) full and reduced dose FOLFIRINOX – median survival 22.4 versus 22.9 months (p=0.2) and 4) full and reduced dose other NAT: median survival 21.1 versus 18.8 months (p=0.4)

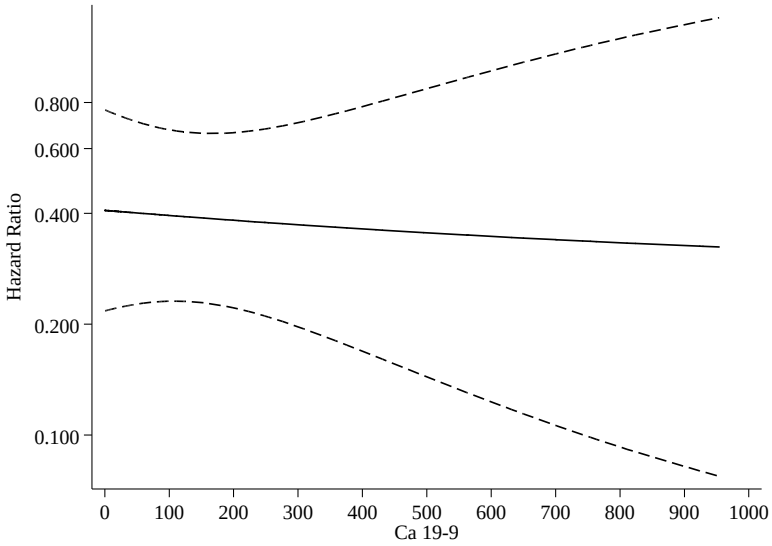


Figure 10: Mortality hazard ratio (line) and 95% confidence interval (dash lines) comparing resected versus non-resected patients as function of Ca 19-9. Data were fitted with a Cox proportional hazard model using restricted cubic splines for Ca 19-9, an interaction between Ca 19-9 and resection and adjusting for age, gender, and type of chemo.

5.3. Study III

Overall 525 resected patients with PC extending in the head and neck of the pancreas were included - 402 with resectable PC, 69 with BRPC, and 54 with LAPC. The groups differed in the proportion of patients receiving NAT – 0.2% among resectable, 25% among BRPC and 87% among LAPC patients ($p<0.0001$), but similar proportion received adjuvant CHT (61%, 64%, and 62% respectively, $p=0.8$). More patients in the resectable and BRPC group had elevated preoperative CA19-9>200 compared to the LAPC group (44% versus 25%, $p=0.01$). Also, more patients in the resectable and BRPC group had elevated preoperative mGPS of 1/2 than in the LAPC group (28% versus 11%) ($p=0.005$). The 30-day and 90-day mortality was 1.7% and 3.2%, and similar among primary resectable, BRPC, and LAPC groups ($p=0.3$). Significantly more patients in the BRPC group had PALN+ (n=13, 27%) compared to the LAPC group (n=4, 9%, $p=0.03$).

As shown in study I and II, there was no significant difference in survival among patients with primary resectable, BRPC, and LAPC: median survival of 20, 15 and 17 months ($p=0.31$, Figure 11).

mGPS could be calculated in 518 patients - 381 patients (73.6%) had mGPS 0 and 137 patients (26.4%) had GPS 1/2. Patients with mGPS of 0 had better expected survival than patients with mGPS of 1/2, as there was no difference in survival among resectable, BRPC and LAPC groups for the scores of 0 and 1/2 (Figure 12). Correcting for possible compromised mGPS did not change the survival estimates. After NAT, though, this observed association of mGPS with survival disappeared and did not seemed to follow the same pattern: the 1-, 3- and 5- year survival was 62% and 83%, 38% and 63%, and 27% and 63% ($p=0.15$).

Preoperative serum CA19-9 was available in 492 patients. Elevated Ca19-9>200 was observed in 205 patients (42%). Elevated CA19-9>200 U/mL compared to ≤200 was associated with significantly impaired survival (figure 13A). The survival among patients with resectable, BRPC, and LAPC was not different neither for Ca 19-9≤200 (data not shown), nor for with elevated Ca19-9>200 (Figure 13B).

PALN were reported in 447 patients and were found positive for metastases in 78 of them (17.4%). Positive PALN showed the strongest association with impaired survival. The median, 1-,3-,5-year survival of patients with positive or negative PALN was 11 and 24 months and 40 and 76%, 12% and 36%, and 0 and 19%, respectively ($p<0.0001$, Figure 14A). There was no difference in the survival among patients with resectable, BRPC, and LAPC neither in the case of PALN–negative scenario, nor in PALN-positive situation (Figure 14B). For each tumor category, though, resectable, BRPC or LAPC, the survival was significantly impaired if there were metastases to PALN.

Preoperative mGPS score did not correlate with the presence of positive PALN: 16% in patients with mGPS 0 and 21% in patients with mGPS 1/2 ($p=0.1549$). Elevated Ca19-9>200, though, showed a significant association with PALN+ disease: 22% compared to 14% in patients with CA19-9≤200 ($p=0.0381$). Combining elevated CA19-9 and mGPS of 1/2 did not increase the chance for encountering PALN+ compared to CA19-9≤200 and mGPS 0: 22% (n=12/55) versus 12% (n=21/172). However, only in the group receiving NAT, elevated CA19-9>200 was associated with significantly increased risk for finding PALN+: 36% (4/11) versus 3% (1/34, $p=0.01$). Adjuvant CHT was able to improve the survival of resected PALN+ patients, but yet the longer-term prognosis was significantly inferior, compared to patients with PALN-, receiving adjuvant CHT (Figure 15).

When the preoperative factors were combined into all positive and all negative groups, this dichotomization selected poor and favorable survivors, particularly in the primary resectable group (Figure 16).

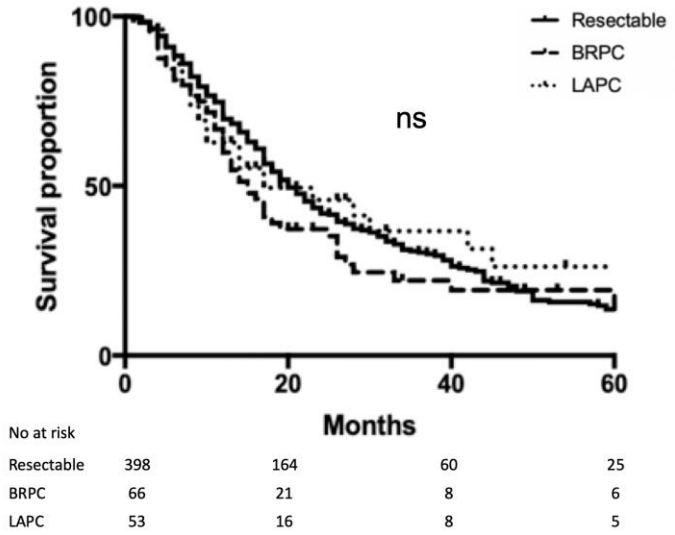


Figure 11: Kaplan-Meier survival probability of patients with resectable (solid line), BRPC (dashed line), and LAPC (dotted line). The median, 1-, 3-, and 5-year survival was 20, 15, and 17 months and 70%, 60%, 63%; 30%, 22%, 37%%; and 14%, 19%, and 26%, respectively (p=0.3075, ns).

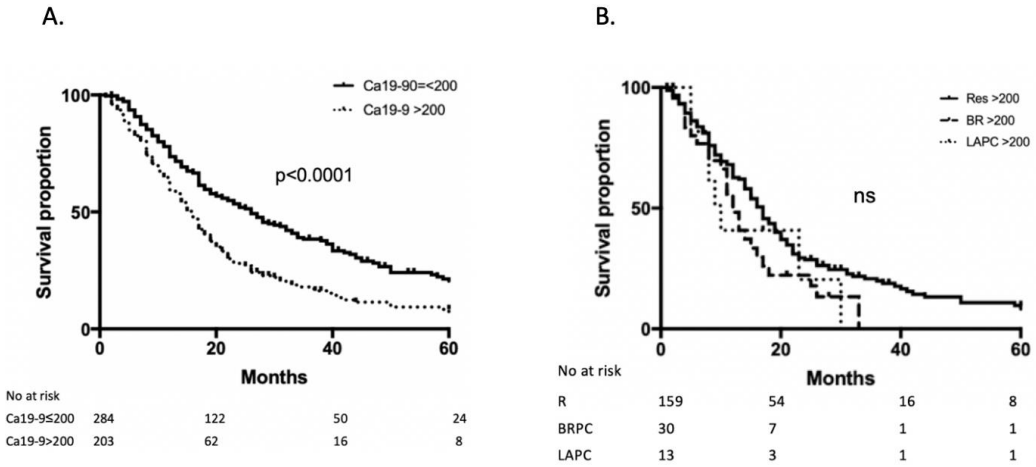


Figure 13: Kaplan-Meier survival analysis of patients according to serum Ca19-9 values. **A**. Overall survival of patients with Ca19-9 ≤200 (solid line) or >200 U/mL (dotted line): the median, 1-, 3-, and 5-year survival was 26 and 16 months and 78% and 65%; 38% and 17%; 21% and 8%, respectively (p<0.0001). **B.** Survival of patients with Ca 19-9 >200 U/mL with resectable (solid line), BRPC (dashed line), and LAPC (dotted line): the median, 1-, 3-, and 5-year survival was 17, 12, and 10 months; 63%, 48% and 41%; 20%, 0, and 0, and 10%, 0, and 0 (p=0.11).

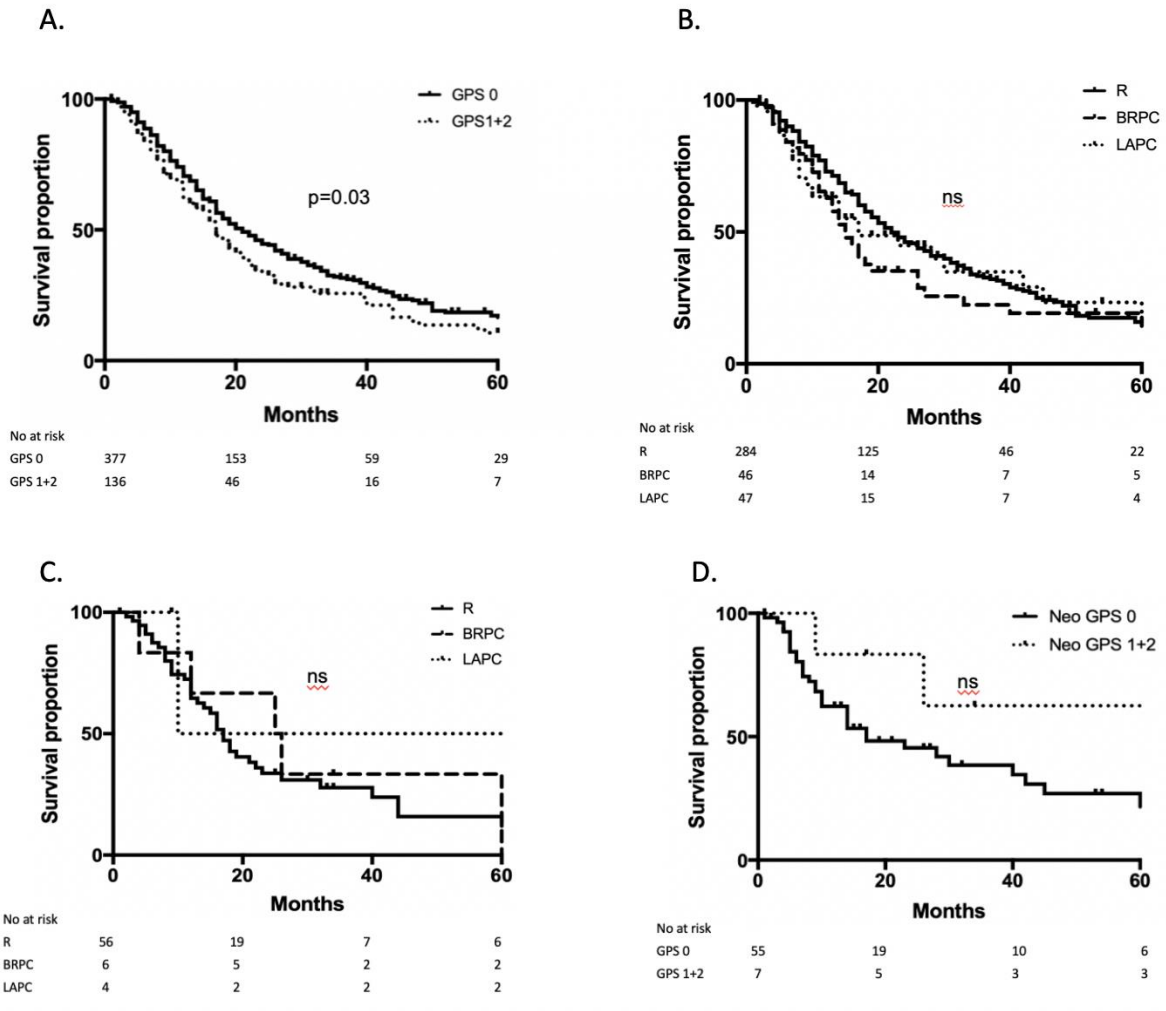


Figure 12: Kaplan-Meier survival probability of patients with mGPS of 0 (solid line) or 1 and 2 (dotted line). **A**. Overall survival. The median, 1-, 3-, and 5-year survival was 21 and 17 months, 71% and 62%, 32 and 26%, 17% and 11 %, respectively (p=0.03). **B.** Overall survival of patients with mGPS 0 with resectable (solid line), BRPC (dashed lined) or LAPC (dotted line). The median, 1-, 3-, and 5-year survival was 22, 15, and 17 months, 73%, 63%, 63%; 33%, 22%, 35%, and 16%, 19%, and 23%, respectively (p=0.24, ns). **C**. Survival of patients with resectable (solid line), BRPC (dashed line) and LAPC (dotted line) with mGPS 1/2 after adjusting for compromised values. The 1-, 3-, and 5-year survival was 66%, 67%, and 50%; 28%, 33%, and 50%; and 16%, 33%, and 50%, respectively (p=0.63, ns). **D.** Survival of patients after NAT with preoperative mGPS of 0 (solid line) or 1/2 (dotted line): 1-, 3-, and 5-year survival of 62% and 83%, 39% and 63%, and 27% and 63%, respectively (p=0.15, ns).

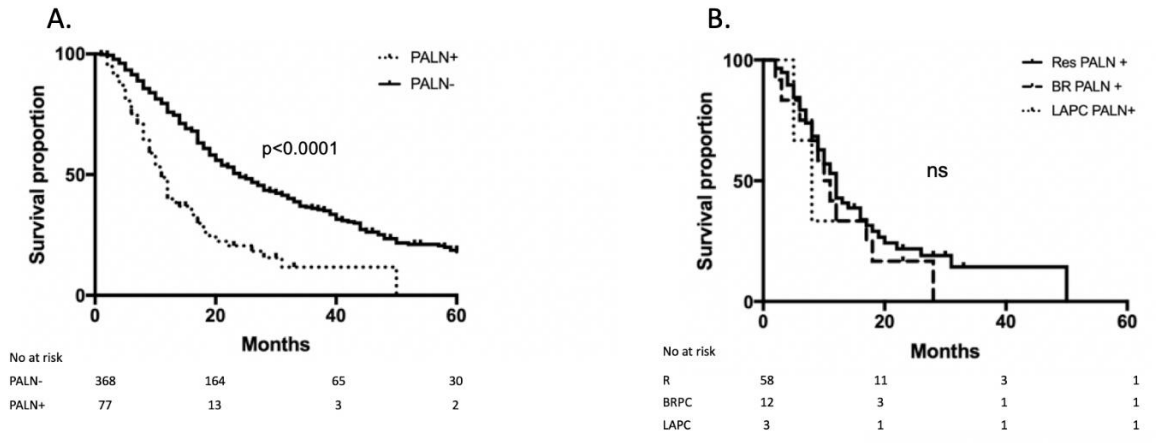


Figure 14: Kaplan-Meier survival analysis of patients according to PALN status. **A.** Survival of patients with PALN (+) positive (dotted line) and PALN (-) negative (solid line) lymph nodes. The median, 1-, 3-, and 5-year survival was 11 and 24 months and 40 and 76%, 12% and 36%, and 0 and 19% (p<0.0001). **B.** Survival of patients with PALN+ nodes, with resectable PC (solid line), BRPC (dashed line), and LAPC (dotted line). The median, 1-, 3-, and 5-year survival was 12, 10.5, and 8 months and 43%, 33%, 33%; 14%, 0%, 33%; and 0, 0, and na, respectively (p=0.6382).

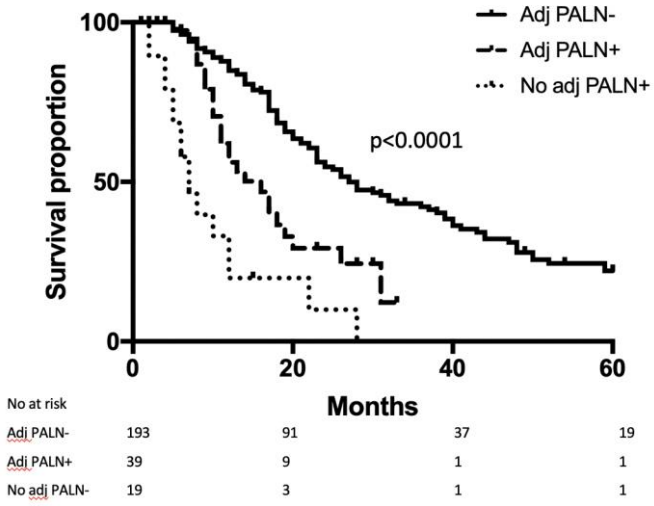


Figure 15: Kaplan-Meier survival analysis of patients with PALN- receiving adjuvant chemotherapy (solid line) and PALN+ receiving adjuvant chemotherapy (dashed line) or no adjuvant therapy (dotted line). The median, 1-, 3-, and 5-year survival was 27, 16 and 7 months; 85%, 56% and 20%; 42%, 12% and 0%, 22%, na, 0, respectively (p<0.0001).

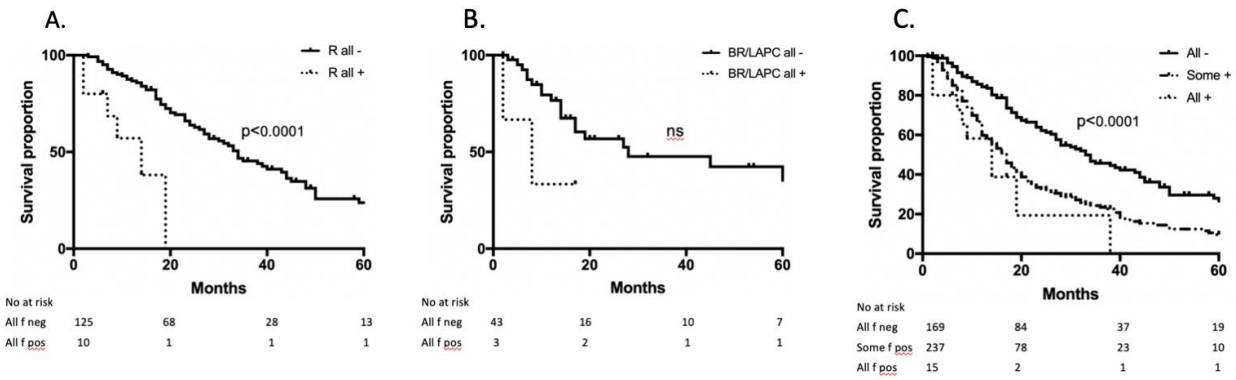


Figure 16: Kaplan-Meier survival analysis of patients if all three risk factors were "negative" (mGPS 0, Ca19-9≤200, and PALN-, solid line), all "positive" (sCa19-9>200, mGPS 1/2, PALN+, dotted line) or in between with only some positive factors. **A.** Survival in patients with primary resectable PC: the median, 1-, 3-, and 5-year survival in "negative" and "positive" patients was 34 and 14 months; 87% and 57%; 45% and 0, and 24% and 0, respectively (p<0.0001). **B.** Survival in patients with BR/LAPC: the median, 1-, 3-, and 5-year survival in "negative" and "positive" patients was 28 and 8 months; 77% and 33%; 48% and 33%; and 42% and n.a. (p=0.1267). **C.** Survival in all patients with PC with "negative", some positive (dash-dotted line) and all "positive" factors: the median, 1-, 3-, and 5-year survival was 33, 17, and 14 months; 84%, 60%, 58%; 47%, 23%, 19%; and 28%, 10%, 0, respectively (p<0.0001).

5.4. Study IV

Tissue specimen from 17 patients operated for suspected PDAC (confirmed in 15 patients) were used for analysis, whereas 4 of the specimens were obtained by small Tru-cut® biopsies. Using the cytokine cocktail IL-2/IL-15/IL-21, TILs could be isolated from all 17 patients and expanded to 10^{10}. The TIL phenotype was determined after 4 weeks of expansion (Table 4). The median frequency of $CD3^+CD4^+$ was 34.1% and of $CD3^+$ $CD8^+$ - 54.4%. Five of 17 cultures exhibited >90% $CD8^+$ TILs. The differentiation/maturation phenotype of the TIL lines was examined (Figure 17). TILs (all $CD4^+$, $CD8^+$ and double negative) did reside predominantly in the central memory ($CCR7^+$ $CD45RA^-$) and effector memory ($CCR7^-$ $CD45RA^-$) subsets.

Analysis of the activation/exhaustion TIL cell markers revealed low frequency of 4-1BB, CTLA-4, and $TIM3^+$ in $CD8^+$, $CD4^+$ and $CD4^-CD8^-$ (Figure 18). PD-1+ T-cells were found in 23.9% among the $CD3^+$ $CD4^+$ TILs and in 36.2% among the $CD3^+CD8^+$ TILs. $LAG3^+$ T cells were frequent among $CD8^+$ TILs (96.3%), but found only in 1.8% among $CD3^+CD4^+$ TILs.

Analysis of the TCR Vβ families in TILs showed that some of the Vβ families are preferentially expressed for individual patients (Table 5). Some of the expanded Vβ families showed clonal TCRs – tested by CDR3 TCR length analysis (Table 6, Figure 19) and then TCR sequencing (Table 7).

After phenotyping, the TILs were tested for reactivity (expressed by IFNγ production) to commonly shared TAAs - mesothelin, survivin, and NY-ESO-1 peptides, after 3 days of stimulation. TIL-reactivity was then blocked by the pan-anti-MHC class I Ab W6/32 and HLA-DR-directed antibody L243 (Table 8). Some of the TIL lines exhibited TAA recognition expressed by intracellular cytokine staining (Figure 20).

Most importantly, reactivity against autologous tumor cells was tested. Two tumor cell lines could be established (from Panc 9 and Panc 17) and tested whether it would be recognized by TILs. In the latter case, strong cytotoxicity by TILs against tumor cells was observed, as tested by Cr51 release essay (Figure 21A). In the former case, 99.2% of TIL TCR was monoclonal – belonging to the Vβ13.2 family. TILs exhibited IFNγ production against freshly harvested autologous tumor-cell suspension cells, that could be blocked by anti-MHC-I

antibody (W6/32), but not by anti-HLA-DR (Figure 21B). That shows that an adequate antigen presentation takes place and, in this way, an effective anti-tumor response could be elicited.

Table 4. TILs Cell Population Phenotype

Patient ID	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+CD8^+$	$CD4^-CD8^-$
Panc 1	99.9	4.33	89.3	2.33	4
Panc 2	99.9	56.1	29.8	2.76	11.3
Panc 3	99.8	17.2	71.4	1.21	10.1
Panc 4	99.6	50.5	32.1	2.3	15.1
Panc 5	99.5	99	0.073	0.51	0.39
Panc 6	99.7	41.1	51.3	3.09	4.47
Panc 7	99.7	6.57	89.9	2.62	0.93
Panc 8	99.7	32.8	64.9	1.09	1.24
Panc 9	94.8	2.07	97.2	0.3	0.47
Panc 10	98.6	92.2	5.75	0.6	1.47
Panc 11	99.7	2.24	96.7	0.3	0.76
Panc 12	99.2	71.8	22.9	4.12	1.17
Panc 13	91.7	70.9	24	3.01	2.06
Panc 14	99.9	0.66	95.7	0.13	3.55
Panc 15	99.1	75.9	19.5	3.09	1.47
Panc 16	99.3	34.1	54.4	8.6	2.86
Panc 17	91.8	17.3	65.1	2.92	14.6
Median	99.6	34.1	54.4	2.33	2.06

Frequencies of the different T-cell population was evaluated in TIL. Frequencies are represented from the parental population (ie, frequency of $CD3^+$ cells in TIL, frequency of $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and $CD4^-CD8^-$ T-cells in the $CD3^+$ cell population).

TILs indicate tumor-infiltrating lymphocytes.

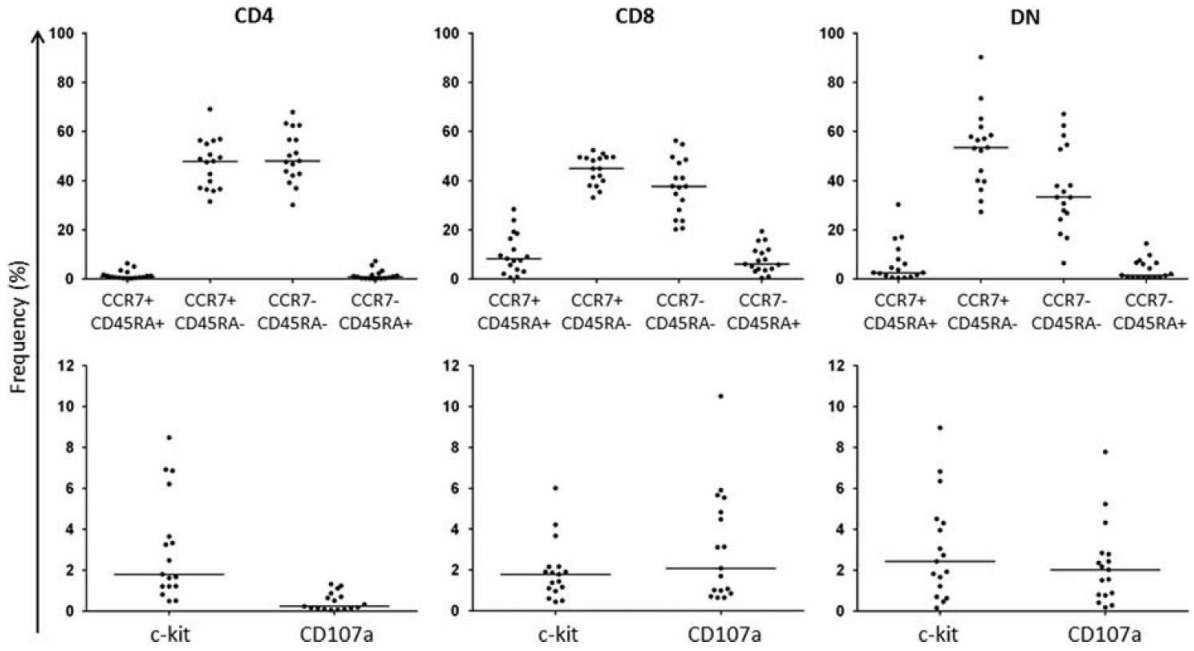


Figure 17: Frequencies of the T-cell populations defined by memory phenotype markers (CD45RA and CCR7) (top) and c-kit and CD107a expression in $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ (bottom).

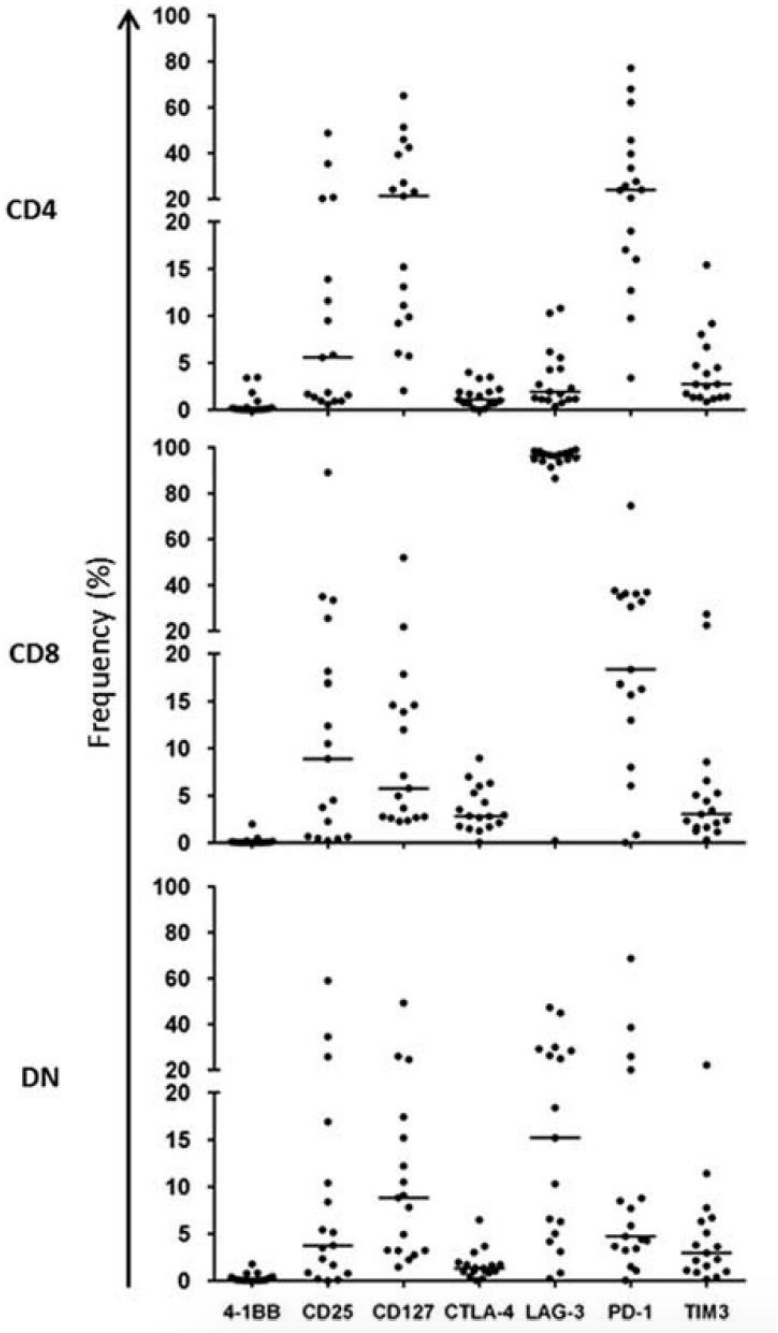


Figure 18: Frequency of activation and exhaustion markers (CD25, CD127, 4-1BB, PD-1, TIM3, LAG-3, and CTLA-4) in CD4+, CD8+, and CD4-CD8- TILs (each dot corresponds to an individual patient).

Table 5: Frequency of TCR Vβ families in $CD4^+$ and $CD8^+$ TILs (the preferentially expanded families are highlighted)

	Panc 1		Panc 2		Panc 3		Panc 4		Panc 5		Panc 6		Panc 7		Panc 8		Panc 9	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
Vβ1	2,8	77,1	3,2	2,3	3,4	0,8	0,2	2,1	1,5	0,2	0,7	1,1	0,3	0,1	1,5	1,2	0,6	0,1
Vβ2	8,0	0,6	9,8	2,6	4,8	0,2	8,5	2,0	0,0	0,0	9,6	19,9	0,1	0,7	7,4	1,7	12,0	0,0
Vβ3	2,1	0,2	4,8	1,9	26,7	0,5	4,8	8,3	0,0	6,1	4,2	0,3	5,1	28,7	2,4	12,0	1,0	0,0
Vβ4	0,4	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,0	3,2	0,0
Vβ5.1	0,4	0,4	0,7	0,5	0,3	0,0	0,1	0,0	0,0	0,3	9,0	0,3	41,1	6,2	9,3	0,0	3,6	0,1
Vβ5.2	0,2	0,1	1,5	0,0	1,5	0,0	0,1	0,2	0,2	0,0	0,2	13,7	2,4	0,2	0,3	0,2	0,1	0,1
Vβ5.3	0,1	0,1	1,6	0,4	1,9	0,0	0,0	0,0	0,0	0,0	0,3	0,1	0,0	0,9	0,6	0,1	0,0	0,0
Vβ7.1	0,2	0,3	0,7	2,1	0,9	3,4	0,2	0,1	0,1	0,0	0,1	0,1	0,1	0,2	5,1	0,3	0,6	0,0
Vβ7.2	6,6	0,0	0,3	0,1	0,9	1,7	0,2	0,1	0,0	0,0	1,1	0,1	1,0	0,4	5,3	3,8	13,9	0,0
Vβ8	1,9	1,7	3,7	0,6	2,9	0,1	0,4	0,4	42,7	4,1	1,2	0,3	5,4	0,5	2,3	2,2	0,5	0,1
Vβ9	1,6	0,0	0,8	0,3	0,3	0,1	0,1	0,1	40,5	0,1	1,7	0,0	0,2	0,1	1,0	0,1	4,8	0,0
Vβ11	0,5	0,0	0,5	0,2	1,5	0,0	0,1	0,7	0,3	0,0	0,6	2,3	4,0	1,0	0,2	0,0	1,0	0,1
Vβ12	3,2	0,9	1,9	0,9	2,5	0,1	0,7	1,8	0,0	0,0	0,6	0,3	0,0	0,0	0,1	0,1	0,4	0,0
Vβ13.1	2,4	0,3	4,6	10,4	1,5	0,5	2,6	4,2	0,0	0,1	0,8	0,0	8,1	10,0	5,6	16,5	5,0	0,0
Vβ13.2	3,1	0,2	1,4	0,4	1,8	0,2	0,2	0,1	0,0	0,0	0,2	0,6	0,3	1,1	0,3	0,0	0,5	99,2
Vβ13.6	2,8	9,1	3,6	0,5	1,2	0,1	0,2	0,1	0,2	0,1	0,2	0,0	1,7	0,1	3,2	6,9	0,4	0,0
Vβ14	1,9	1,4	2,0	3,6	1,3	63,3	0,4	0,7	0,0	0,0	1,0	5,3	3,2	2,8	0,6	1,4	0,4	0,1
Vβ16	2,4	1,0	6,9	6,9	3,3	0,3	4,1	1,1	0,0	0,1	0,8	0,0	0,1	0,3	0,1	25,4	0,5	0,0
Vβ17	4,6	0,1	2,9	1,8	0,8	0,0	0,2	0,0	0,0	0,0	1,5	0,0	1,0	32,9	1,3	0,1	12,4	0,0
Vβ18	2,4	0,1	0,2	0,0	0,5	0,0	0,1	0,0	0,4	0,0	0,4	0,2	1,0	0,2	3,6	0,0	0,4	0,0
Vβ20	7,0	5,9	6,6	13,8	4,6	0,1	3,7	0,1	0,0	0,4	0,7	0,2	0,1	0,0	1,4	0,4	0,1	0,0
Vβ21.3	0,4	0,5	3,8	4,3	0,4	0,1	0,1	0,1	0,0	3,2	6,2	0,1	2,4	0,0	0,6	0,3	0,5	0,0
Vβ22	11,1	0,2	3,3	1,7	2,8	0,2	6,2	1,8	0,1	0,0	15,1	2,1	0,2	2,6	1,4	0,6	1,9	0,7
Vβ23	3,7	0,3	0,4	0,1	0,8	0,8	0,9	0,5	1,6	0,5	0,1	4,6	0,1	0,1	0,2	6,7	0,1	0,0

	Panc 10		Panc 11		Panc 12		Panc 13		Panc 14		Panc 15		Panc 16		Panc 17	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
Vβ1	4,4	7,1	13,0	0,5	1,4	25,8	0,1	0,3	0,9	16,2	0,6	2,8	0,3	0,4	2,2	5,7
Vβ2	4,1	39,8	2,4	0,2	7,5	4,8	1,5	0,6	5,1	0,7	29,3	1,5	48,9	1,0	16,3	1,1
Vβ3	3,7	1,3	0,0	0,0	1,7	8,8	0,0	0,0	13,3	2,0	1,1	17,9	11,7	0,2	17,8	2,6
Vβ4	6,0	0,1	0,0	0,0	2,2	0,4	4,8	0,0	0,9	0,1	8,3	1,2	0,1	0,1	0,0	0,0
Vβ5.1	10,8	1,1	0,6	0,7	3,4	0,5	4,6	0,2	2,7	0,8	4,2	1,6	15,2	0,2	7,7	0,0
Vβ5.2	1,3	9,4	0,1	0,1	0,3	1,8	0,1	0,1	0,4	0,1	2,3	0,8	1,2	0,1	0,4	0,0
Vβ5.3	0,7	18,0	0,2	0,0	0,3	1,6	0,1	0,0	0,1	0,3	4,2	0,3	0,9	0,0	1,4	0,4
Vβ7.1	0,5	1,5	0,0	0,0	1,6	2,8	0,1	0,3	0,3	6,3	0,9	1,8	0,3	2,2	1,1	1,5
Vβ7.2	1,6	0,1	0,0	0,0	0,4	0,1	0,0	0,0	0,7	4,8	0,4	0,1	0,0	0,0	2,2	1,4
Vβ8	2,7	1,4	0,4	0,3	3,6	1,8	1,1	0,5	1,7	0,8	1,9	4,1	0,5	0,3	2,7	1,9
Vβ9	3,4	1,2	0,5	0,2	6,7	1,0	2,4	0,1	9,7	2,3	2,0	2,0	1,8	0,4	1,9	1,5
Vβ11	0,6	0,3	0,1	0,2	0,8	0,3	0,2	0,2	0,3	0,2	1,4	0,7	0,4	0,2	0,2	0,0
Vβ12	1,6	0,5	0,0	0,0	0,3	1,0	0,3	0,0	1,5	5,9	0,3	0,2	0,3	0,1	4,8	0,7
Vβ13.1	3,7	0,1	35,5	0,1	9,2	0,3	0,5	0,2	4,3	19,6	0,7	0,8	2,6	1,1	1,5	0,9
Vβ13.2	6,2	0,7	0,2	0,0	2,5	1,4	0,2	0,2	4,4	0,9	1,8	23,0	0,4	0,6	3,2	3,4
Vβ13.6	2,0	0,3	0,6	0,0	1,5	0,2	0,5	0,2	0,3	1,9	0,6	0,9	0,3	0,4	1,1	2,0
Vβ14	3,6	0,8	0,1	0,0	0,7	3,5	0,1	0,3	2,9	2,5	0,9	7,2	2,1	0,6	1,6	7,6
Vβ16	1,0	0,2	0,0	0,2	0,1	0,1	0,0	0,2	0,2	0,8	0,2	0,2	0,1	0,0	1,3	0,0
Vβ17	7,5	0,3	0,1	12,3	3,3	2,3	31,1	0,9	9,9	1,4	1,3	1,9	1,5	0,5	0,3	0,1
Vβ18	0,5	0,2	0,1	0,2	0,3	0,3	0,5	0,1	0,9	3,1	3,3	3,6	0,4	3,3	0,3	0,0
Vβ20	2,5	0,5	0,1	0,1	1,4	0,2	0,0	0,0	0,7	0,3	0,5	0,6	0,0	0,8	1,3	4,0
Vβ21.3	2,4	0,1	0,9	0,0	0,5	8,0	0,5	0,0	1,2	0,5	0,4	0,4	0,2	1,6	1,5	6,8
Vβ22	3,4	1,0	3,6	0,1	5,2	2,0	26,0	65,9	3,6	0,8	0,7	2,9	6,2	6,4	2,6	0,2
Vβ23	4,5	0,7	0,6	0,1	0,2	0,4	0,3	1,0	0,5	0,7	1,8	7,4	0,3	0,3	1,1	2,0

Table 6: Vβ families and clonality in TILs after expansion.

Patient	Cell population	Family by pcr	Vβ by Flow cytometry	Number of clones
Panc 1	CD8	TRBV9	Vβ1	2
Panc 5	CD4	TRBV12	Vβ8	1
Panc 7	CD4	TRBV5,1	Vβ5,1	1
	CD8	TRBV19	Vβ17	2
		TRBV28	Vβ3	2
Panc 8	CD8	TRBV14	Vβ16	1
Panc 9	CD8	TRBV6	Vβ13,2	1
Panc 10	CD8	TRBV20,1	Vβ2	2
Panc 11	CD8	TRBV20	Vβ2	1
		TRBV7,3/6/7/9	No antibody	1
		TRBV19	Vβ17	1
Panc 13	CD4	TRBV19	Vβ17	1
Panc 14	CD8	TRBV9	Vβ1	2
Panc 15	CD4	TRBV20,1	Vβ2	2
	CD8	TRBV6,2	Vβ13,2	2
Panc 16	CD4	TRBV20,1	Vβ2	1
	CD8	TRBV7,3	No antibody	2
		TRBV7,2,8	No antibody	2
		TRBV19	Vβ17	1

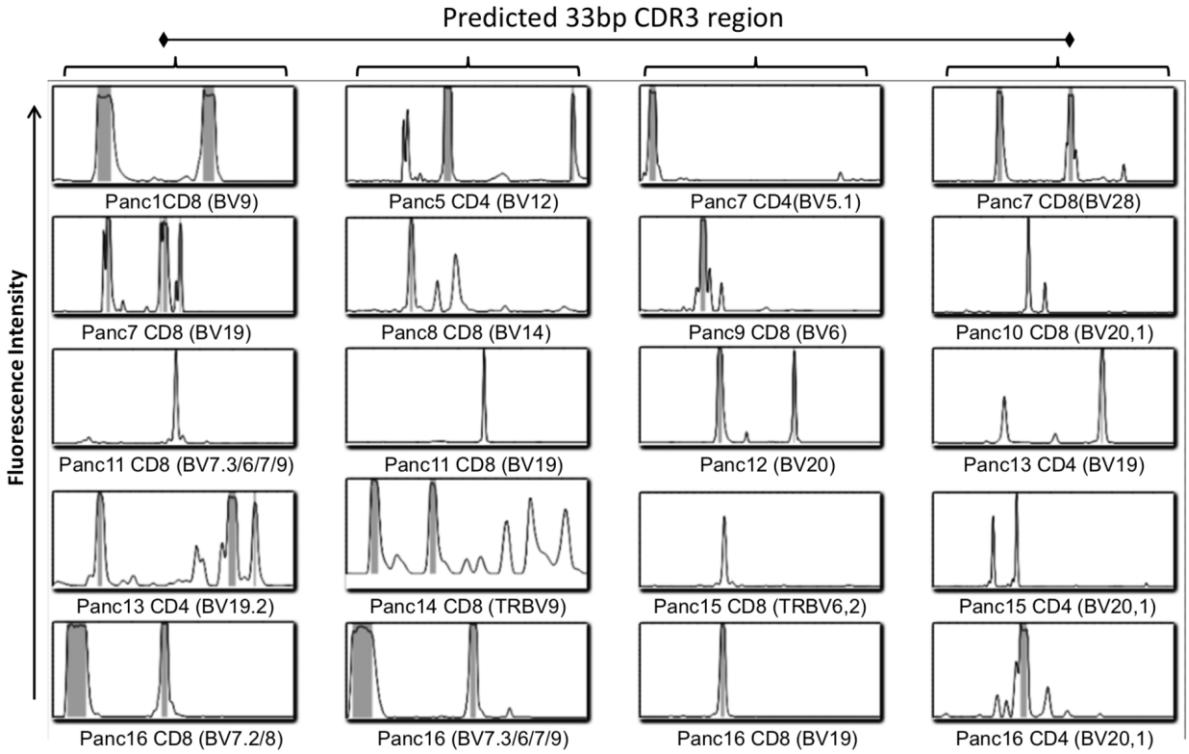


Figure 19: TCR CDR3 analysis of frequent TCR Vβ families after 4-week expansion with IL-2, IL-15, and IL-21.

Table 7: CRD3 sequence analysis in TIL.

TIL ID	Cell subset	Vβ	Vβ	(-D-)	Jβ	Jβ	Flow antibody
Panc 1	**CD8**	TRBV9	CAS	SVQGVSQ	ETQYF	TRBJ2-5	Vβ1
Panc 5	**CD4**	TRBV12-3/4	CAS	SRRGSG	DTQYF	TRBJ2-3	Vβ8
Panc 7	**CD4**	TRBV5.1	CAS	STDSN	TEAFF	TRBJ1-1	Vβ5.1
	CD8	TRBV28	CAS	SFQTAHT	DTQYF	TRBJ2-3	Vβ3
Panc 8	**CD8**	TRBV14	CAS	SLRDS	DEAFF	TRBJ1-1	Vβ16
Panc 9	**CD8**	TRV6-2/3	CAS	SLQGRV	DEQFF	TRBJ2-1	NA
Panc 16	**CD4**	TRBV20-1	CSA	RDGTLN	TEAFF	TRBJ1-1	Vβ2
		TRBV20-1	CSA	RPLRDRVA	HGYTF	TRBJ1-2	Vβ2
	CD8	TRBV19	CAS	SILAFRAG	ETQYF	TRBJ2-5	Vβ17
		TRBV7-6	CAS	SQGPN	YEQYF	TRBJ2-7	NA
Panc 17	**CD8**	TRBV7-2	CAS	SFGLAGA	NEQFF	TRBJ2-1	NA
		TRBV7-2	CAS	SSRLAGTY	NEQFF	TRBJ2-1	NA

Table 8. TAA IFNγ production (pg/mL) in TIL in response to commonly shared TAAs (only positive results are shown).

	NY-ESO-1	NY-ESO1 + W6/32	NY-ESO1 + L243	Survivin	Survivin + W6/32	Survivin + L243	Mesothelin	Mesothelin + W6/32	Mesothelin + L243
Panc 1	6.97	0	0	0	0	0	262.23	15.26	0
Panc 2	24.20	0	0	0	0	0	291.27	13.50	0
Panc 10	4.13	0	0	66.06	0	0	78.70	72.88	0
Panc 11	0	0	0	0	0	0	131.16	0	0
Panc 14	10.47	0	0	0	0	0	275.15	nd	249.95
Panc 16	173.19	0	0	8.35	0	0	19.64	0	60.84

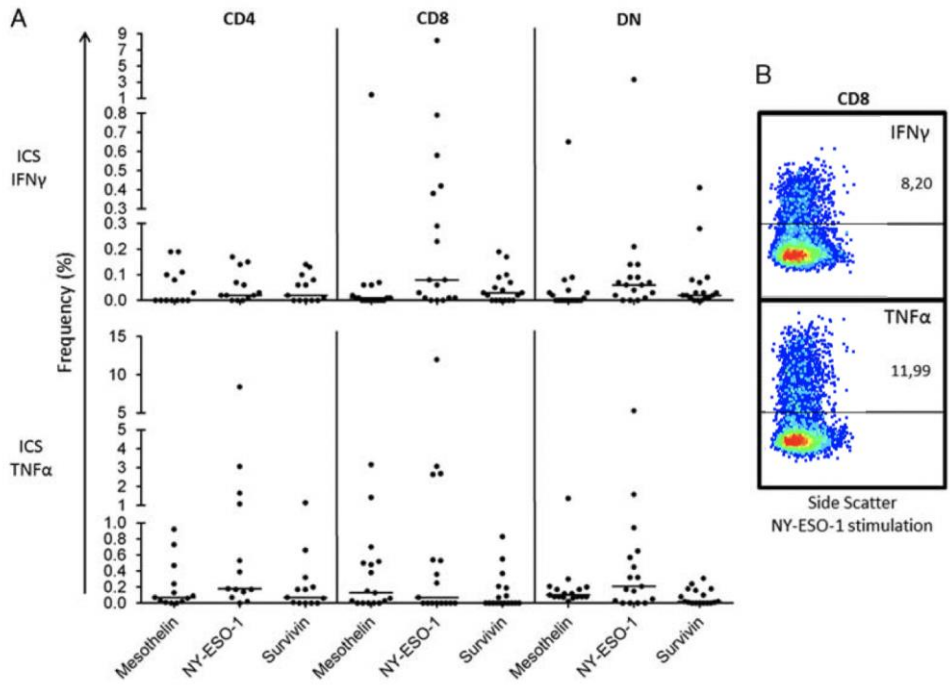


Figure 20: A. TAA recognition analysis by intracellular cytokine staining (IFNγ and TNFα) in TIL lines (dots represent individual patients, medium values are substracted). B. ICS analysis of IFNγ and TNFα production after NY-ESO-1 stimulation in CD8+ cells from Panc 6 TILs.

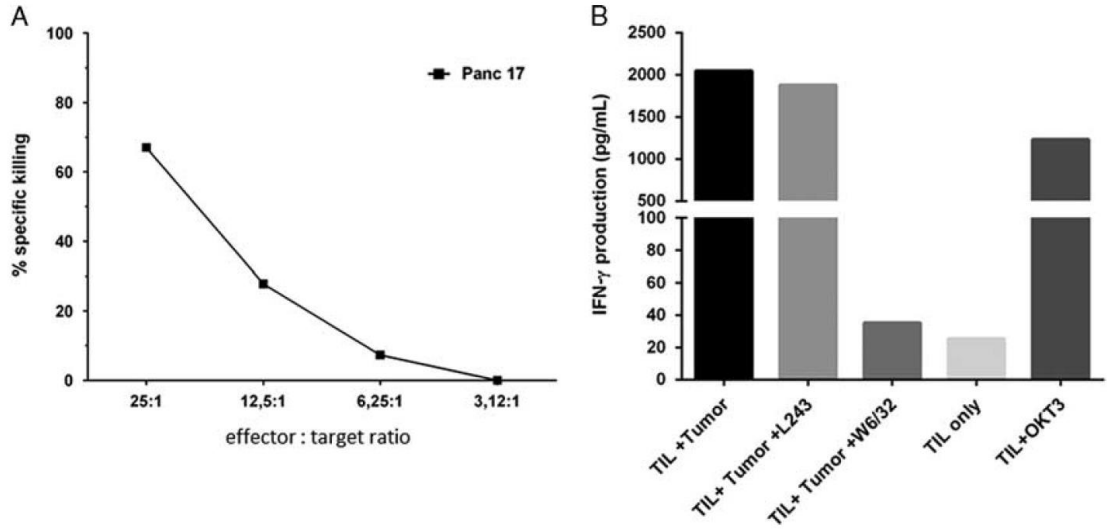


Figure 21: A. Recognition by TIL of the autologous tumor cell line in Panc 17 by standard Chromium 51 release essay. B. IFNγ production in TILs after co-culture with autologous with autologous tumor cells (Panc 9). $CD8^+$ TILs (Vβ13.2 dominant) recognize autologous tumor; IFNγ production can be inhibited by the anti-MHC-class I antibody (W6/32), but not with the anti-HLA-DR-directed mAb.

6. Discussion

6.1.Study I

This is the largest separately reported series on VR with pancreatectomy from a single-center, with outcome based on uniform surgical strategy. It confirms that VR can be carried out safely. DGE dominated the surgical complication spectrum, but had no different rate even in the standard surgical resection. The reason for the high incidence does not lie in the specific complication profile of VR, but is rather a consequence of the perioperative management, maybe not the least on the quite liberal early oral feeding allowance policy. The reoperation rate of 8% was much lower than what was reported in previous single-center studies (24%)(142). The 90-day mortality was also low, and actually two of the deaths were due to rapid tumor progression and not due to surgical complications (both in patients with primary resectable tumors, without NAT).

The study is the only single-center series that digests in detail the thrombosis rate after VR and the possible causes for it. A much lower thrombosis rate than previously described was reported (4.5 versus 7.5 to 26.7%)(177, 183, 185) at the same time as the general other thromboembolic events occurred with a rate of 8.3%, revealing that safe reconstruction can be achieved with meticulous preoperative technique and perioperative management. Interestingly, thrombosis rate after DP was much higher. Possibly, leaving the pancreatic head in place puts the reconstruction under tension – in all cases a direct anastomosis was performed.

Thus, this large series indicates clearly, that with good surgical practice venous reconstruction can safely be performed, irrespective of patients' characteristics or local tumor involvement, so it should be performed whenever necessary to obtain radicality. The study eliminates previous speculations and uncertainties regarding the safety of the venous resections and the choice of surgical technique, where the data generally came from large series integrating the results of several small centers. Also, perhaps keeping the venous reconstruction technique simple, with a segmental resection and one primary anastomosis after a good mobilization with a Cattell-Braasch maneuver, shortens the time for venous cross clamping, maintains geometrically even contours allowing for laminar blood flow and avoids morbidity associated with harvesting a venous interponate from a different anatomic location.

The survival of patients undergoing VR for IPMN cancer and distal cholangiocarcinoma, was statistically not different, but definitely not inferior. Other than PDAC tumors are generally excluded from survival analysis, to obtain homogeneous groups, and these have not been addressed properly as to what the surgical strategy should be, despite that assumingly VR might offer them also survival advantage. Thus, VR should be to considered whenever necessary to obtain radicality, irrespective of the origin of the diagnosed periampullary tumor.

As reported previously, the survival after VR was similar to that with standard resections (142). No study has systematically reported before what the expected survival is after VR if the pancreatic tumor was primary resectable, BRPC or LAPC. The study showed that the survival was not statistically significant among the groups. The survival of LAPC patients appeared surprisingly greatly superior, although not statistically significant, possibly due to the relatively small number of patients. This finding lifts up the question whether NAT should be considered even in the groups of patients with any extent of the suspected venous involvement, even primary resectable. Due to its retrospective observational design, the study could not answer this question. To penetrate better the role of radical resection, comparison of patients operated with isolated VR and combined arterial-venous resection was performed, and contrary to what has been observed previously, showed no statistically significant difference. Since the number of patients in the combined resection group was relatively small, this is a heavily pretreated group and that this type of resections is not performed in the majority of centers world-wide, but are a subject to the experience of very limited number of surgeons, they were excluded from the final survival analysis, in order to set the focus on procedures that have wide applicability. The type of venous reconstruction did not have an impact on survival either, despite that there very few patients in the type 2 and 4 groups and none of them made it to the 5-year survival bench-mark. There was no difference in survival either according to the four types of venous reconstruction. It seems that an adequate resection to obtain sufficient resected length that guarantees radicality and as simple and maintain the anatomic geometry reconstruction provides even the best long-term outcome results.

On multivariate survival analysis, interestingly, none of the factors influencing survival were technical in nature (dealing with surgical technique or technically difficult place of infiltration). Factors related to patients' general health and reflecting tumor biology seem to

play much bigger role than anticipated, despite that none of these are part of the classification systems. Metastatic disease, exclusively represented by metastases to PALN, had the the strongest relation to impaired survival on univariate analysis, but probably due to the relatively few patients, failed to reach statistical significance on multivariate analysis. Despite that this association has been repeatedly observed, the topic of whether patients with metastases to PALN should be excluded from upfront resection is still controversial. Apparently, new guidelines should be considered to give more adequate recommendation reflecting the patients' chance for survival and hence in what order the therapeutic modalities should be applied to them.

6.2.Study II

The second study investigated a series of patients with BRPC/LAPC, receiving NAT and considered for surgical resection after obtaining stable disease or downsizing of the tumor and the extent of vascular involvement. A strength of the study was that it encompassed a non-selected patient population and thus revealed that in real-time circumstances only 2/3 of the patients were able to receive the planned NAT, due to various reasons – inability to obtain biopsy, patient deterioration or very rapid disease progression. Despite its superiority above other treatment regimen, FOLFIRINOX could be administered only to 34.6% of the patients. Thus, the study highlights what could realistically be expected in clinical practice and sets the frame to investigate whether there might be alternative regimens with equivalent survival effect of combined surgical-oncologic approach. Significant dose reductions and drug-combination modification were not unusual seen either, so the determination of what impact they might have on survival was crucial.

The proportion of patients requiring extended resection was much higher than generally reported, at the same time as the morbidity profile was not different than standard resections despite the surgical aggressiveness. At the same time all patients who progressed had distant metastases and very few – local recurrence, even in the locally advanced group. This fact underlines that aggressive approach is indeed feasible and should be carried out whenever there are good signs that the pancreatic disease is under local control.

A major finding, as in study I, was that the survival among pre-treated patients with BRPC and LAPC was not statistically different, neither for general group, nor for the surgically resected patients. This once again confirm that in the setting of NAT and radical surgical approach, the current classifications of local tumor involvement do not make sense. What really makes a difference for the prognosis was whether the patients could be radically resected.

Comparing resected and non-resected groups in a decent time-frame is difficult. We chose the starting time point for survival as the date of diagnosis, since thus the whole survival can be encompassed and this is what matters from the patients' perspective. Statistically, though, the study was thus prone to immortal time bias. This is inevitable, considering the retrospective character of the studies and there is no flawless way to correct for it. Two strategies could be generally used. One is to define the starting date as the date when the planned first treatment is discontinued. However, the strategy is unrealistic for the patients who do not survive the initial treatment. Another way is to define the starting point as date of diagnosis and the second treatment as a time-dependent indicator variable taking a value of 0 or 1. The disadvantage of this strategy is that patients' characteristics are not equivalent when the decision for treatment "2" has been taking. Patients who do not receive the second treatment have generally more aggressive disease responding with either progression or distant spread to therapy "1". The most adequate way would probably be to compare patients with stable disease who can or cannot be resected (treatment "2") due to purely technical reasons, whenever there is no possibility for safe reconstruction. However, the number of patients was not enough by the time the study was planned and an analysis was done later, including more patients treated with NAT and undergoing surgical exploration. Macroscopically during surgery, the resected and non-resected patients had no evidence of distant metastases. Further on, the para-aortic lymph nodes (station 16b1), was examined in the majority (not all) patients and confirmed negative, which is as far we can reach today, confirming M0 stage of disease was present. Still patients undergoing surgical resection had clear survival benefit

From a biological standpoint of view, however, what might be considered a flaw in study design and a source of bias, it is probably more correct to be defined as desired selection criteria of who might benefit from a local therapeutic approach that surgery is. Rather than correcting for it, one should emphasize on how to better select patients as having a uniform ground to enter into prospective therapeutic trials.

Looking at the different NAT regimens, the study did not a statistically significant difference in survival between the resected patients treated with FOLFIRINOX or any other type of combination chemotherapy. It is worth noticing, though, the 5-year survival after FOLFIRINOX was 46.2% compared to 27.6% after other combination therapies. Both, the observed survival probabilities are undoubtedly superior than what is generally observed in primary resectable PDAC with up-front resection, which is in a way paradoxical. It highlights the idea that NAT might be able to have a major impact on survival even in patients with resectable PDAC.

Interestingly, significant dose reductions of NAT, most often expressed by giving less than 80% of the planned dose and less than 80% of the planned cycles, did not have a significant impact on survival when followed by resection. In this context, patients with stable disease should not be excluded from exploration in case the intended chemotherapy dose was not reached. Particularly for FOLFIRINOX, significant dose reductions did not have impact on survival. This finding is substantial since it is not unusual that FOLFIRINOX is denied to patients supposing they will not fulfill the planned treatment with concern about serious toxic effects. Being able to modify the regimen to avoid side effects without jeopardizing its efficacy might increase its applicability to a much larger cohort of patients. Whether 80% dose in FOLFIRINOX should be considered standard and what is the threshold that the treatment combination loses its efficacy, would need to be validated in larger cohorts.

Interestingly, regarding the preoperative values of CA19-9, we did not find any cut-off when the patients lost the benefit of surgical resection. Rather, surgery kept its association with improved survival even if the mortality rate slightly increased for higher CA19-9 levels. Thus, even patients with higher levels of Ca 19-9 should not be definitely excluded from resection attempt. Whether they should be considered good responders to NAT, just because the radiologic tumor involvement did not change or more likely would benefit for switch to other oncologic treatment needs to be further assessed.

6.3.Study III

The study investigated the impact of known preoperative risk factors on the survival of patients with pancreatic cancer involving the head and neck regions of the pancreas. Once again, this study confirmed that there was no difference in survival among patients with primary resectable, BRPC, and LAPC although there was a difference in the proportion of patients receiving NAT. More patients in the resectable and BRPC group had elevated CA19-9, higher mGPS scores and PALN+ than in the LAPC group, implying that the administration of NAT might attenuate the presence of these risk factors and imply its possible utility even in primary resectable PC.

Although elevated mGPS scores showed association with impaired survival, the segregation effect was not that impressive and there were long-term survivors in both groups. Due to missing values of preoperative CRP or albumin, meaningful distinction between score 1 and 2 could not be made as too much patient data would have been sacrificed. Thus, a more possible stronger predictive effect of score of 2 on survival might have been lost. So far, studies estimating the predictive value of mGPS after NAT in LAPC patients to possible better select the candidates for surgery, are practically lacking. Unfortunately, this study showed that after NAT, the association of mGPS with survival seems to disappeared and have paradoxical effects. Possibly NAT induces inflammation as potential sign of efficacy and activated immune response instead of the detrimental tumor inflammation, that is the core of this phenomenon.

Elevated CA19-9 gave also a much better survival estimate than what current classifications for local tumor involvement can achieve. For every subcategory (resectable PC, BRPC, LAPC), it was able to differentiate between better and poorer survivors. There is no uniform consensus as to what cut-off should be set and consequently whether it should be used as a firm break-point for decisions towards surgery, NAT or therapy switch. Setting too low value as a cut-off might lose some of its predictivity, particularly as false positive results are not uncommon. With too high value, many patients with possibly worse prognosis might have been missed. The value of 200, previously validated as a good separation point, seems to capture well the survival probability (195).

PALN + status was confirmed to have strongest impact om survival as the survival among resectable, BRPC, and LAPC in case of PALN + or PALN – situation, was similar. To our knowledge, this is the first report demonstrating this correlation. Elevated Ca19-9 >200 was associated with higher risk for PALN+ encounter – at least in every 5 patients. This observation was reported in one smaller study previously in resectable patients (253). Combining elevated CA19-9 with elevated mGPS scores did not improve the PALN+ detection yield in any group. Interestingly, in the subgroup of patients receiving NAT, elevated CA19-9 showed an even stronger correlation to the presence of PALN – in every 3 patients, while NAT itself did not have a beneficial effect on survival in PALN+ patients. This is to indicate that elevated CA19-9 should presumably be considered a failure for NAT to reach its effect and would indicate at least a switch in treatment before any attempt for surgical resection. Elevated CA19-9 may by itself be a sufficient marker, by attempt to confirm the PLAN status by biopsy may not be unreasonable. The number of patients with elevated CA19-9 after NAT were too few in this study to make a meaningful subgroup comparison. However, a trend was observed for even worse survival in patients with PALN+ compared to PALN- in patients with CA19-9>200. Thus, it is not certain that LAPC patients after NAT with PALN+ have any benefit from resection.

Adjuvant therapy has previously been shown to improve the prognosis of resected PALN + patients. That was confirmed in the current study, but anyhow the survival in the group was much inferior than the survival of patients with PALN- receiving adjuvant CHT. More reasonably, the efforts should be directed towards neoadjuvant oncologic treatment if a better prognosis is to be reached.

Combining all three risk factors was able to distinctly dichotomize all patients into poor and good survivors. The difference was impressive for the primary resectable, not pre-treated group. That separation could possibly be a marker for whom could be selected for upfront surgery and who would need a multimodality approach. For the LAPC group, it seems that CA19-9, without mGPS is a sufficient marker.

It is certain that the current technical classification based solely on the radiologic suspicion of major involvement does not reflect tumor biology and can hardly be used to make decisions on therapeutic algorithm – oncologic treatment or surgical resection. Better survival estimate can be achieved by standard known preoperative prognostic factors to identify high-risk

patients for expected shorter survival that might require multimodality treatment approach before the decision for resection is taken. The current tumor classifications need to be revised, implementing the known preoperative risk factors in order to be able to generate more accurate survival estimates as a prerequisite for development of better therapeutic algorithms.

6.4.Study IV

TILs could be isolated from all 17 patients and expanded to a sufficient amount 10^{10}, that could be a prerequisite for cellular therapy. A bit longer culture times were necessary to obtain TILs from smaller biopsies, yet still in good amount for possible adoptive transfer. The combination cocktail of cytokines IL-2/IL-15/IL-21 seemed to provide a better premise for that than classic IL-2, used to expand TILs, as it may lead to activation-induced death and older TILs. “Younger” TILs usually demonstrate better persistence and tumor-reactivity and the central-memory T cells (CR45RA-CCR7+) phenotype that has better premise for homing and effector functions. Possibly the combination of IL-2/Il-15/IL-21 in the culture medium is able to selectively drive the expansion of this phenotype. The cytokine cocktail might potentially drive toward Th1 responses derived from Th2 tumor environment.

The best scenario would be to test the TILs for their recognition and reactivity to autologous tumor cells. Expanding autologous tumor cells in PC is, though, a very difficult task and we succeeded only twice in it. Therefore, TILs were tested for commonly expressed TAAs, like mesothelin, survivin, and “strong” antigens, like NY-ESO-1. In individual TIL lines it was possible to detect strong reactivity against these antigens, that could be blocked by mAbs to MHC class I and II antigens. This is also an evidence that adequate antigen presentation takes place and, which is the best premise for specific T cell activation and effector responses. TILs might target also not only cancer cells, but also stromal cells and this phenomenon needs to be further explored. It is possible that targeting cancer cells and fibroblasts may result in epitope spreading and induction of more potent specific response – something that has been described in malignant melanoma.

For phenotyping the isolated TILs, it was important to find out whether they express activation and exhaustion markers – an event occurring the they have “met” and recognized

an antigen. Such markers could be detected, meaning the TILs are not randomly present in the tumor but are selected to persist due to specific recognition. Interestingly, although PD-1 is most often regarded as an exhaustion marker, its presence on T cells is evidence that these are "experienced" and have recognized an antigen, possibly a tumor antigen. Being able to demonstrate that TILs TCRs belong only to some Vβ families is also a very strong link to oligoclonality. This was confirmed by PCR and sequencing of the TCR showing that actually most of the time the TILs recognize private, specific for the tumor mutations.
The tumor- cell-specific killing demonstrated at last, of course has the disadvantage that it happens in the absence of the extracellular matrix that would possible at least to some extent hamper the TIL penetrance and contact with tumor cells. However, the desired tumor cell death is a fact. The higher the ratio between the TILs to tumor cells – the more pronounced the effect, which highlights the significance of expanding significant number of TILs for cellular therapy.

7. Conclusions

Venous resection during pancreatectomy, should be considered whenever possible in all types of periampullary tumors, irrespective of tumor origin or radiologic local disease involvement.

Specific complications to VR are rare. The place and type of VR/reconstruction do not influence outcome and should be tailored to the patients' anatomic characteristics.

Complex surgery for LAPC is feasible. Surgical resection for LAPC, in the multimodality setting, provides a good chance for long term survival.

FOLFIRINOX brings survival benefit in LAPC potentially even in larger dose reductions. Other combination chemotherapy might be an alternative, but full doses should be attempted.

Current classifications on resectable, BRPC, and LAPC do not reflect the patients' chance for survival with the current therapeutic strategies and need to be revised.

Elevated CA19-9 identifies much better patients with worse survival, irrespective of local disease extension, and needs to be integrated into the prognostic systems.

Metastatic PALN are the strongest predictors worse survival in both resectable, BRPC, and LAPC. Elevated CA19-9 implies higher risk for PALN+ detection.

Combination of elevated CA19-9, mGPS, and PALN+ identifies the group with worst survival in resectable PC.

In LAPC after NAT, CA19-9 alone is highly predictive of PALN+ presence and worse survival.

TILs from PC can be successfully cultured even from small biopsies in sufficient amount to be potentially used for therapy.

TILs can recognize TAAs and autologous cancer cells in an MHC class I-restricted manner and induce tumor killing in culture.

8. Future Perspectives

This thesis validates the shift of concept that even locally advanced pancreatic cancer can be considered a surgical disease and that complex pancreatic surgery is feasible and can be done with good results. It also points out the necessity to more widely consider multimodality approach, but to do that adequately, factors indicating high-risk for disease progression need to be better appreciated. Hence, the necessity to update the current classification systems, so they reflect better the patients' chances for survival.

First of all, extensive work is ongoing from clinical and administrative prospective to firmly establish the program for the structured treatment of locally advanced pancreatic cancer. Although the frame has been set, wider impact has not been yet achieved. Thus, more patients would have access to interdisciplinary evaluation and a possibility for surgical resection, and potentially a chance for cure.

There is an ongoing interinstitutional call to gather and reconsider the current classification systems on local involvement from a survival point of few. To set the ground for that, a few proposals to join institutional databases in order to better appreciate the biologic risk factors are on the way.

More observational studies are ongoing to evaluate the correlation between radiologic morphologic tumoral factors and biomarkers and their relationship to cancer survival. Prospective randomized trials are under design stage and ongoing – to evaluate whether NAT may improve the prognosis in patients with primary resectable cancer or after further risk stratification.

The basic science work is ongoing to better understand the role of the immune system in pancreatic cancer – about the interrelationship among TILs, fibroblasts and cancer cells, and their impact on survival. Despite that the translational TIL project has temporary on hold, the premise is present to restart even the phase I and II immunology trials, in collaboration or own institution.

9. Populärvetenskaplig sammanfattning

En kirurgisk operation är den enda behandlingen som kan leda till bot hos patienter med bukspottkörtelcancer. På grund av detta är klassifikationssystemen som bedömer hur långt kommen tumören är rätt ”tekniska” i sin karaktär. De bedömer sannolikheten för kirurgisk borttagning av tumören, men avspeglar inte helt tumörens biologi, vilket säger hur aggressivt tumören kan växa och sprida sig oavsett storlek.

Patienter som har tumörer med övergripande växt har hittills haft lika dåliga chanser att överleva som patienter med spridd sjukdom. Då ”starkare” cellgiftsbehandlingar börjar bli tillgängliga är det viktigt att reda ut huruvida en cellgiftskombinationsbehandling tillsammans med mer omfattande kirurgi (som kräver kärlrekronstruktioner) kan ge bättre utfall. Då denna behandling kan innebära högre risker är det väsentligt att kunna bedöma vilka som kan ha nytta av den. Därav bör klassifikationssystemet bättre avspegla chansen till överlevnad. Utöver detta är det ytterst nödvändigt att leta efter andra mer biologi inriktade behandlingsmöjligheter för att bekämpa den aggressiva naturen av tumörerna.

Syftet med denna avhandling är att reda ut huruvida klassifikationerna är tillräckligt bra för att bedöma överlevnadsmöjligheterna vid de behandlingar som finns idag, eller om de kan göras bättre. Avhandlingen vill reda ut vilka tekniskt kirurgiska metoder som är säkrast och ger bäst utfall. Här identifieras även de faktorer som ytterligare måste tittas på för att ge rätt behandling till de patienter som idag har sämst utfall.

Avhandlingens första och andra studie visa att mer avancerad kirurgi utförs säkert på sjukhus som har stor erfarenhet av denna kirurgi. Här påvisas att tekniska faktorer så som utformning av tumören, var den växer eller hur man kopplar kärlen, ej påverkar de kirurgiska resultaten eller chansen till överlevnad om man använder god kirurgisk metodik. Större tumörer med avancerad växt kan opereras bort efter cellgiftsbehandling med åtminstone lika bra överlevnadsresultat som operationer av små tumörer. Starka cellgifter i kombination ger bättre resultat än en enkel behandling. Även om inte hela dosen cellgifter har kunnat ges samtidigt som tumören fortfarande tekniskt kan opereras bort, är det värt att försöka då chansen att överleva sjukdomen är bättre än om man avstår.

Faktorer som kan mätas i blodet avspeglar bättre tumörens aggressivitet än dess morfologiska omfattning. Dessa faktorer samt patienternas allmänhälsa kan bättre bedöma hur stor överlevnadschansen är. Därav kan även patienter med små tumörer ha nytta av cellgiftsbehandling före operation.

De tre första studierna som presenteras i avhandlingen visar att med behandlingen som finns idag så är klassifikationssystemet av tumörer inte tillräckligt för att visa chansen till överlevnad. Det finns faktorer i blodet som borde användas regelmässigt för att styra vilken behandling som skall användas.

I avhandlingens fjärde och sista studie utforskas möjligheten att odla fram patienternas egna immunceller (”vakterna” i kroppen) från tumören. Dessa ”tränas” sedan att känna igen och bekämpa tumören för att sedan återföras till patienten. Denna typ av immunbehandling har givit imponerande resultat hos andra typer av tumörer, men har ännu inte tagits fram för bukspottkörtelcancer. Denna studie visade att med en ny metod kan tillräckligt många av dessa immunceller odlas fram för en behandling även om lite vävnad fanns tillgänglig. Dessa celler visar tecken på att de kan se den specifika tumören och att de inte finns där av tillfälle. I en odling kan immuncellerna identifiera tumörceller, reagera starkt, och till sist döda tumörcellerna.

Sammanfattningsvis så visar avhandlingen att under rätt behandlingsordning så kan fler patienter med bukspottkörtelcancer opereras och få chans till bot. Det finns andra faktorer än hur tumörerna ser ut som måste beaktas för att bättre identifiera de högriskpatienter som eventuellt behöver annan behandling innan eventuell kirurgi. Dessa faktorer borde ingå i det klassifikationssystem som bedömer hur biologiskt långt gångna tumörerna är. Det finns dessutom hopp om att en ny behandling kan etableras som använder kroppens egna förmåga att besegra cancern.

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